



METHODS FOR DETERMINING WHETHER AN AGENT POSSESSES A DEFINED BIOLOGICAL ACTIVITY

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of Provisional Application No. 60/442,797,
5 filed January 24, 2003, and Provisional Application No. 60/474,413, filed May 30, 2003.

FIELD OF THE INVENTION

The present invention relates to methods for screening biologically active agents,
such as candidate drug molecules, to identify agents that possess a defined biological
activity.

10 BACKGROUND OF THE INVENTION

Identifying new drug molecules for treating human diseases is a time consuming
and expensive process. A candidate drug molecule is usually first identified in a
laboratory using an assay for a desired biological activity. The candidate drug is then
tested in animals to identify any adverse side effects that might be caused by the drug.
15 This phase of preclinical research and testing may take more than five years. *See, e.g.,*
J.A. Zivin, Understanding Clinical Trials, *Scientific American*, ps. 69-75 (April 2000).
The candidate drug is then subjected to extensive clinical testing in humans to determine
whether it continues to exhibit the desired biological activity, and whether it induces
undesirable, perhaps fatal, side effects. This process may take up to a decade. *Id.*

20 Adverse effects are often not identified until late in the clinical testing phase when
considerable expense has been incurred testing the candidate drug. There is a need,
therefore, for methods that increase the likelihood of identifying candidate drugs that
possess a desirable biological activity, and which do not cause adverse side effects, early

in the testing process, thereby reducing the amount of time and resources expended during drug testing.

SUMMARY OF THE INVENTION

5 In accordance with the foregoing, in one aspect the present invention provides methods for determining whether an agent possesses a defined biological activity. Each method of this aspect of the invention includes the steps of: (a) making at least one comparison from the group consisting of: (1) comparing an efficacy value of the agent to at least one reference efficacy value to yield an efficacy comparison result, wherein each efficacy value represents at least one expression pattern of the same efficacy-related
10 population of genes, or at least one expression pattern of the same efficacy-related population of proteins; (2) comparing a toxicity value of the agent to at least one reference toxicity value to yield a toxicity comparison result, wherein each toxicity value represents at least one expression pattern of the same toxicity-related population of genes, or at least one expression pattern of the same toxicity-related population of proteins; (3)
15 comparing a classifier value of the agent to at least one reference classifier value to yield a classifier comparison result, wherein each classifier value represents at least one expression pattern of the same classifier population of genes, or at least one expression pattern of the same classifier population of proteins; and (b) using the comparison result(s) obtained in step (a) to determine whether the agent possesses the defined
20 biological activity.

The methods of this aspect of the invention can utilize one, two, or all three of the foregoing comparisons identified by numbers (1), (2) and (3). In embodiments of the invention that utilize two or three of the foregoing comparisons, the comparisons can be made in any temporal sequence (*e.g.*, in embodiments of the invention that utilize all
25 three of the foregoing comparisons, comparison (1) can be made before or after comparison (2), and before or after comparison (3)). Optionally, the methods of this aspect of the invention can include the step of first identifying one or more of the efficacy-related population of genes or proteins, toxicity-related population of genes or proteins, and/or classifier population of genes or proteins. The foregoing populations of
30 genes or proteins can be identified, for example, by using the methods disclosed herein for identifying an efficacy-related population of genes or proteins, a toxicity-related population of genes or proteins, and/or a classifier population of genes or proteins.

In some embodiments of the methods of this aspect of the invention, the defined biological activity is the ability to affect a biological process *in vivo*, and at least one of the efficacy value of the agent, the toxicity value of the agent and the classifier value of the agent is/are calculated from gene expression levels, and/or protein expression levels, measured in living cells cultured *in vitro*. In some embodiments of the methods of this aspect of the invention, the defined biological activity is the ability to affect a biological process in a first living tissue, and at least one of the efficacy value of the agent, the toxicity value of the agent and the classifier value of the agent is/are calculated from gene expression levels, and/or protein expression levels, measured in a second living tissue, wherein the first living tissue is a different type of tissue than the second living tissue.

The methods of this aspect of the invention are useful in any situation in which it is desirable to know whether an agent possesses a defined biological activity in a living thing (*e.g.*, prokaryotic cell, eukaryotic cell, plant or animal). For example, the methods of this aspect of the invention are useful in the preclinical stage of drug discovery to identify chemical agents that possess a desired biological activity (*e.g.*, a biological activity that ameliorates the symptoms of a disease), but which elicit few, if any, undesirable side effects when administered to a living organism, such as to a human being or other mammal.

In another aspect, the present invention provides populations of nucleic acid molecules that are useful in the practice of the methods of the present invention as probes for measuring the level of expression of members of a classifier population of genes, or an efficacy-related population of genes, or a toxicity-related population of genes, wherein the classifier population of genes, the efficacy-related population of genes, and the toxicity-related population of genes are each useful for identifying agonists, or partial agonists, of PPAR γ . In a related aspect, the present invention provides classifier populations of genes, efficacy-related populations of genes, and toxicity-related populations of genes that are useful in the practice of the methods of the invention for identifying agonists, or partial agonists, of PPAR γ .

In yet another aspect, the present invention provides methods for identifying an efficacy-related population of genes or proteins, methods for identifying a toxicity-related population of genes or proteins, and methods for identifying a classifier population of genes or proteins, as described more fully herein. The methods of this aspect of the

invention are useful, for example, for identifying efficacy-related populations of genes or proteins, toxicity-related populations of genes or proteins, and classifier populations of genes or proteins, that are useful in the practice of the methods of the invention for determining whether an agent possesses a defined biological activity.

5 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press, Plainsview, New York (1989), and Ausubel et al., *Current Protocols in Molecular Biology* (Supplement 47), John Wiley & Sons, 10 New York (1999), for definitions and terms of the art.

In one aspect, the present invention provides methods for determining whether an agent possesses a defined biological activity. The methods of this aspect of the invention each include the steps of: (1) comparing an efficacy value of the agent to at least one 15 reference efficacy value to yield an efficacy comparison result, wherein each efficacy value represents at least one expression pattern of the same efficacy-related population of genes, or at least one expression pattern of the same efficacy-related population of proteins; (2) comparing a toxicity value of the agent to at least one reference toxicity value to yield a toxicity comparison result, wherein each toxicity value represents at least 20 one expression pattern of the same toxicity-related population of genes, or at least one expression pattern of the same toxicity-related population of proteins; (3) comparing a classifier value of the agent to at least one reference classifier value to yield a classifier comparison result, wherein each classifier value represents at least one expression pattern of the same classifier population of genes, or at least one expression pattern of the same 25 classifier population of proteins; and (b) using the comparison result(s) obtained in step (a) to determine whether the agent possesses the defined biological activity.

In the practice of this aspect of the invention, the amounts of nucleic acid gene products (*e.g.*, the amount of mRNA transcribed from a gene, as represented by the amount of cDNA made from the transcribed mRNA) from defined gene populations are 30 measured, or the amounts of proteins in defined protein populations are measured, to yield gene or protein expression patterns that provide information about the effect of an agent on a living thing. It is sometimes desirable to measure protein levels instead of the

levels of gene transcripts because the amount of a protein in a living thing may depend on factors in addition to the level of transcriptional activity of the gene that encodes the protein. For example, the amount of a protein in a living thing may be affected by the activity of a specific protease in a living thing, or on the activity of the protein translational apparatus. These factors may be affected by an agent used to treat a living thing.

As used herein, the term "agent" encompasses any physical, chemical, or energetic agent that induces a biological response in a living organism *in vivo* and/or *in vitro*. Thus, for example, the term "agent" encompasses chemical molecules, such as candidate therapeutic molecules that may be useful for treating one or more diseases in a living organism, such as in a mammal (*e.g.*, a human being). The term "agent" also encompasses energetic stimuli, such as ultraviolet light. The term "agent" also encompasses physical stimuli, such as forces applied to living cells (*e.g.*, pressure, stretching or shear forces).

The term "biological activity" refers to the ability of an agent to affect (*e.g.*, stimulate or inhibit) one or more biological processes in a living organism. Examples of biological processes include biochemical pathways; physiological processes that contribute to the internal homeostasis of a living organism; developmental processes that contribute to the normal physical development of a living organism; and acute or chronic diseases.

As used herein, the phrase "efficacy value" refers to a value that numerically represents the level of expression, in response to an agent, of one of the following: (1) all of the genes within an efficacy-related population of genes; or (2) all of the proteins within an efficacy-related population of proteins.

As used herein, the phrase "efficacy-related population of genes" refers to a population of genes, present in a living thing, that yields at least one expression pattern, in response to an agent, that correlates (positively or negatively) with the presence of at least one desired biological response caused by the agent in the living thing.

As used herein, the phrase "efficacy-related population of proteins" refers to a population of proteins, present in a living thing, that yields at least one expression pattern, in response to an agent, that correlates (positively or negatively) with the presence of at least one desired biological response caused by the agent in the living thing.

As used herein, the phrase "toxicity value" refers to a value that numerically represents the level of expression, in response to an agent, of one of the following: (1) all of the genes within a toxicity-related population of genes; or (2) all of the proteins within a toxicity-related population of proteins.

5 As used herein, the phrase "toxicity-related population of genes" refers to a population of genes, present in a living thing, that yields at least one expression pattern, in response to an agent, that correlates (positively or negatively) with the presence of at least one undesirable biological response caused by the agent in the living thing.

10 As used herein, the phrase "toxicity-related population of proteins" refers to a population of proteins, present in a living thing, that yields at least one expression pattern, in response to an agent, that correlates (positively or negatively) with the presence of at least one undesirable biological response caused by the agent in the living thing.

15 As used herein, the phrase "classifier value" refers to a value that numerically represents the level of expression, in response to an agent, of one of the following: (1) all of the genes within a classifier population of genes; or (2) all of the proteins within a classifier population of proteins.

20 As used herein, the phrase "classifier population of genes" refers to a population of genes, present in a living thing, that yields at least two different gene expression patterns caused by at least two different agents. One of the two expression patterns correlates (positively or negatively) with the presence of a first biological response caused by one of the at least two agents. Another of the at least two expression patterns correlates (positively or negatively) with the presence of a second biological response, that is different from the first biological response, caused by another of the at least two agents. Thus, a classifier population of genes is used to classify an agent into one or more classes based upon the expression pattern of the classifier population of genes that is induced by the agent.

25 As used herein, the phrase "classifier population of proteins" refers to a population of proteins, present in a living thing, that yields at least two different protein expression patterns caused by at least two different agents. One of the two expression patterns correlates (positively or negatively) with the presence of a first biological response caused by one of the at least two agents. Another of the at least two expression patterns correlates (positively or negatively) with the presence of a second biological response,

that is different from the first biological response, caused by another of the at least two agents. Thus, a classifier population of proteins is used to classify an agent into one or more classes based upon the expression pattern of the classifier population of proteins that is induced by the agent.

5 Representative Biological Activities: The methods of this aspect of the invention are useful in any situation in which it is desirable to know whether an agent possesses a defined biological activity in a living thing. The term "living thing" encompasses all unicellular and multicellular organisms (*e.g.*, plants and animals, including mammals, such as human beings), and also encompasses living tissue, and living organs.

10 The term "biological activity" can refer to a single biological response, or to a combination of biological responses. Representative examples of biological activities include stimulation or suppression of one or more of the following biological processes that affect the concentration of glucose in mammalian blood: uptake, transport, metabolism and/or storage of glucose by living cells. Further representative examples of
15 biological activities include stimulation or suppression of one or more of the following biological processes that affect the concentration of cholesterol in mammalian blood: stimulation or suppression of cholesterol uptake by living cells, and/or cholesterol metabolism by living cells, and/or cholesterol synthesis by living cells. Again by way of non-limiting example, the methods of the invention can be used to identify agents that
20 affect (*e.g.*, stimulate, or inhibit) one or more of the following biological processes or disease states: Alzheimer's disease; schizophrenia; cancerous tumor size; body mass index; inflammation; and cell division rate.

 A biological activity can be defined in terms of any measurable effect, or combination of measurable effects, of an agent on a living thing. For example, a
25 biological activity can be defined with reference to stimulation, and/or inhibition, of one or more biological responses; and/or the absolute and/or relative magnitude of stimulation, and/or inhibition, of one, or more, biological responses; and/or the inability to affect (*e.g.*, the inability to stimulate or inhibit) one, or more, biological responses.

 Thus, for example, a defined biological activity can be the ability to stimulate a
30 target biological response (*e.g.*, raise the level of high density lipoprotein in human blood). Again by way of example, a defined biological activity can be the combination of the ability to stimulate a target biological response (*e.g.*, raise the level of high density

lipoprotein in human blood) without stimulating one, or more, undesirable biological responses (*e.g.*, without increasing blood plasma volume, or without causing liver damage). By way of further example, in the context of comparing numerous agents within a population of agents, the defined biological activity can be the combination of causing the strongest stimulation of a target biological response, while causing the least stimulation of an undesirable biological response (*i.e.*, in this example the agent, within the population of agents, that most strongly stimulates the target biological response, but causes the least stimulation of an undesirable biological response, possesses the defined biological activity).

The use of efficacy values in the practice of the invention: The methods of the invention can include the step of comparing an efficacy value of an agent to at least one reference efficacy value to yield an efficacy comparison result, wherein each efficacy value represents at least one expression pattern of the same efficacy-related population of genes, or at least one expression pattern of the same efficacy-related population of proteins. In some embodiments, an efficacy value of the agent is compared to a scale of efficacy values to yield an efficacy comparison result, wherein each efficacy value represents at least one expression pattern of the same efficacy-related population of genes, or at least one expression pattern of the same efficacy-related population of proteins.

An efficacy value is a value that numerically represents the level of expression, in response to an agent, of one of the following: (1) all of the genes within an efficacy-related population of genes; or (2) all of the proteins within an efficacy-related population of proteins. The population of efficacy-related genes, or the population of efficacy-related proteins, yields an expression pattern, and, therefore, an efficacy value, that correlates (positively or negatively) with the occurrence of one or more desired biological response(s) caused by an agent in a living thing. A representative example of a desired effect in a living thing is the return of an abnormal expression pattern of a population of genes, and/or proteins, and/or non-protein molecules, in a diseased organism, to a normal expression pattern that is characteristic of a healthy organism. A representative example of a desired effect in a human being suffering from, or predisposed to, atherosclerosis is reduction in the concentration of total cholesterol in the subject's blood plasma.

The expression pattern of an efficacy-related population of genes or proteins induced by an agent, and, therefore, the efficacy value calculated from the induced gene expression pattern, or protein expression pattern, provides an indication of the extent to which an agent induces one or more desired effect(s) in a living thing. Thus, the effectiveness of an agent at inducing one or more desired effect(s) in a living thing can be compared to the effectiveness of one, or more, other agents at inducing the same desired effect(s) in the same living thing.

It is typically easier, and more readily informative, to compare efficacy values of different agents, than to directly compare the expression patterns induced in an efficacy-related population of genes, or proteins, by the agents. For example, the efficacy value of a candidate inhibitor of a target biological response (*e.g.*, a candidate cell division inhibitor that may be useful for inhibiting the growth of cancerous cells in a mammal) can be compared to the efficacy value of a known inhibitor of the same target, biological, response to determine whether the two efficacy values are similar. If the efficacy value of the known inhibitor is similar to the efficacy value of the candidate inhibitor, then it is inferred that the candidate inhibitor inhibits the target biological response. Again by way of example, in the context of comparing candidate inhibitors of a target biological response to determine which candidate inhibitor exerts the strongest inhibitory effect on the target biological response, the efficacy values of each candidate inhibitor are compared to each other, and it is inferred that the candidate inhibitor that has the numerically largest efficacy value exerts the strongest inhibitory effect on the target biological response.

By way of specific and more detailed example, the comparison of efficacy values may be used to identify agents that stimulate a target biological response (*e.g.*, increase the amount of high density lipoprotein in human blood plasma). For example, a population of genes, or proteins, is identified in a living thing that yield(s) at least one expression pattern that positively correlates with the stimulation of the target biological response by at least one agent that is known to stimulate the target biological response. This is the efficacy-related gene population, or efficacy-related protein population. Living cells that include the efficacy-related gene population, or efficacy-related protein population, are contacted with a candidate agent, and the resulting expression pattern of the efficacy-related gene population, or efficacy-related protein population, is measured,

and an efficacy value calculated therefrom. The efficacy value of the candidate agent is compared to the efficacy value(s) of one or more reference agent(s) that is/are known to stimulate the target biological response, and if the efficacy value of the candidate agent is sufficiently similar to the efficacy value(s) of the reference agent(s), then it is inferred
5 that the candidate agent is a stimulant of the target biological response.

An efficacy-related population of genes, or efficacy-related protein population, can be identified, for example, by contacting a living thing (*e.g.*, living tissue, living organ or living organism), or population of living things (*e.g.*, population of living cells in culture), with an agent that is known to cause a target biological response. A population
10 of genes, or proteins, is identified that yields an expression pattern that correlates (positively or negatively) with the occurrence of the target biological response in response to the agent. This population of genes, or proteins, may be used as the efficacy-related gene population, or efficacy-related protein population, respectively.

In another approach, a diseased organism may be used to identify an efficacy-related population of genes or proteins. Thus, for example, in the context of identifying
15 chemical agents useful for ameliorating the symptoms of a target disease that affects humans, a non-human model organism (*e.g.*, a mouse) is identified that suffers from the target disease, or that suffers from a disease that is similar to the target disease and which is a good experimental model for studying the target disease. The diseased model
20 organism may occur naturally, or may be created by human intervention, such as by a selective breeding program, or by genetic manipulation. For example, the technique of targeted homologous recombination can be used to generate mice in which one or more genes are functionally inactivated. By choosing an appropriate gene to inactivate, the resulting mice may exhibit the symptoms of a disease that afflicts human beings, and may
25 be a useful model system for studying the disease and for identifying candidate chemical agents useful for treating the disease.

A non-diseased organism of the same species as the diseased organism (*e.g.*, a non-diseased mouse) is treated with an agent that is known to ameliorate the symptoms of the target disease, and the expression pattern of a representative population of genes, or
30 proteins, from the treated organism is measured. The expression pattern of the same representative population of genes, or proteins, is measured in the diseased organism, and the expression patterns of the genes, or proteins, are compared to identify those proteins,

or genes that produce transcriptional products (*e.g.*, mRNA molecules), whose amount in the organism is affected (*e.g.*, increased or decreased) by the agent, and which are regulated in the opposite direction in the diseased organism compared to the non-diseased organism (*e.g.*, the level of expression of the genes is higher in a non-diseased organism than in a diseased organism, and the level of expression of the genes is increased, toward the non-diseased level, in the diseased organism in response to treatment with the agent). This population of genes, or proteins, is an efficacy-related population of genes, or an efficacy-related population of proteins, useful in the practice of the present invention for identifying agents that ameliorate the symptoms of the target disease.

Optionally, one of skill in the art may determine that a correlation (positive or negative) exists between the expression pattern of the efficacy-related gene population (or an efficacy-related population of proteins) and the amelioration of one or more symptoms of the target disease, thereby confirming the usefulness of the gene, or protein, population as an efficacy-related gene population, or efficacy-related protein population, in the practice of the methods of the present invention.

Example 1 herein describes the use of a strain of mice (referred to as db/db mice) that exhibit the symptoms of diabetes and are useful as a model experimental system for that disease. The db/db mice are used to identify an efficacy-related population of genes whose transcription is reduced in the db/db mice compared to non-diseased mice, and whose transcription is stimulated by rosiglitazone, which is a drug used to treat diabetes.

For example, an efficacy-related population of genes, or proteins, can be identified in the following manner. Living cells are contacted, *in vivo* or *in vitro*, with an amount of a first reference agent that maximally induces (or maximally inhibits) a target biological response. An example of a method for contacting living cells, cultured *in vitro*, with the first reference agent is addition of the first reference agent to the medium in which the living cells are cultured. Examples of methods for contacting living cells, *in vivo*, with the first reference agent is injection into the bloodstream, or injection into a target tissue or organ, or nasal administration of the first reference agent, or transdermal administration of the first reference agent, or use of a drug delivery device that is implanted into the body of a living subject and which gradually releases the first reference agent into the living body.

In the present example, if an efficacy-related population of genes is being sought, messenger RNA is extracted (and may or may not be purified) from the contacted cells and used as a template to synthesize cDNA or cRNA which is then labeled (*e.g.*, with a fluorescent dye). The labeled cDNA or cRNA is then hybridized to nucleic acid molecules immobilized on a substrate (*e.g.*, a DNA microarray). The immobilized nucleic acid molecules represent some, or all, of the genes that are expressed in the cells that were contacted with the first reference agent. The labeled cDNA or cRNA molecules that hybridize to the nucleic acid molecules immobilized on the DNA array are identified, and the level of expression of each hybridizing cDNA or cRNA is measured and compared to the level of expression of the same cDNA or cRNA species in control cells that were not contacted with the first reference agent, thereby revealing a gene expression pattern that was caused by the first reference agent. The population of genes whose expression is affected by the first reference agent can be used as the efficacy-related gene population, and an efficacy value for the first reference agent can be calculated from the levels of expression of all of the mRNAs within the efficacy-related gene population.

In the present example, if an efficacy-related population of proteins is being sought, some, or all, of the protein is extracted from the contacted cells. The identity and abundance of some or all of the proteins within the extracted protein mixture is determined by any suitable technique, such as mass spectrometry, and compared to the level of expression of the same protein species in control cells that were not contacted with the first reference agent, thereby revealing a protein expression pattern that was caused by the first reference agent. The population of proteins whose expression pattern is affected by the first reference agent can be used as the efficacy-related protein population, and an efficacy value for the first reference agent can be calculated from the levels of expression of all of the proteins within the efficacy-related protein population.

More typically, the foregoing, exemplary, procedure is repeated with one or more additional reference agents that each have the same effect as the first reference agent on the same target biological response (*e.g.*, all the reference agents either induce or inhibit the same target biological response). The gene expression patterns, or protein expression patterns, induced by each of the reference agents are compared, and a population of genes or proteins whose expression is affected by each reference agent, and that correlates with the effect on the target biological response, is identified. The gene or protein expression

patterns caused by each of the reference agents are statistically analyzed to identify the population of genes, or proteins, (within the total population of genes or proteins whose expression is affected by all the reference agents) that produces an expression pattern that most strongly correlates with the occurrence of the target biological response. This population of genes, or this population of proteins, can be used as an efficacy-related gene population, or efficacy-related protein population.

Example 1 herein describes the identification of an efficacy-related population of genes that is useful in the practice of the methods of the invention for identifying agonists and partial agonists of peroxisome proliferator-activated receptor γ (hereinafter referred to as PPAR γ). The peroxisome proliferator-activated receptors are nuclear hormone receptors, activated by fatty acids and their eicosanoid metabolites, that regulate glucose and lipid homeostasis in mammals, such as human beings. The PPAR γ subtype plays a central role in the regulation of adipogenesis and is the molecular target for the 2,4-thiazolidinedione class of antidiabetic drugs (e.g., rosiglitazone). See, e.g., J.L. Oberfield, et al., *Proc. Nat'l Acad. Sci. U.S.A.*, 96:6102-6106 (1999). Undesirable side-effects caused by the 2,4-thiazolidinedione class of drugs includes heart enlargement and an increase in blood plasma volume. Thus, there is a need to identify molecules of the 2,4-thiazolidinedione class that are antidiabetic drugs, but which do not cause these undesirable side effects.

In some embodiments of the methods of the invention, the efficacy-related population of genes or proteins yields at least one efficacy-related expression pattern, in response to an agent, that correlates with the presence of at least one desired biological response caused by the agent in a living thing, wherein the at least one efficacy-related expression pattern appears before the desired biological response. Thus, for example, these embodiments of the methods of the invention are particularly useful for high-throughput screening of numerous drug candidates because it is not necessary to wait for the appearance of the desired biological response in order to identify those drug candidates that possess a defined biological activity.

Representative examples of techniques for identifying and measuring the expression of an efficacy-related population of genes: efficacy-related populations of genes are identified by measuring the amount of transcriptional expression of genes in a living thing (e.g., a living thing that has been contacted with an agent that affects a target

biological response). Gene expression may be measured, for example, by extracting (and optionally purifying) mRNA from the living thing, and using the mRNA as a template to synthesize cDNA which is then labeled (*e.g.*, with a fluorescent dye) and can be used to measure gene expression. While the following, exemplary, description is directed to
5 embodiments of the invention in which the extracted mRNA is used as a template to synthesize cDNA, which is then labeled, it will be understood that the extracted mRNA can also be used as a template to synthesize cRNA which can then be labeled and can be used to measure gene expression.

RNA molecules useful as templates for cDNA synthesis can be isolated from any
10 organism or part thereof, including organs, tissues, and/or individual cells. Any suitable RNA preparation can be utilized, such as total cellular RNA, or such as cytoplasmic RNA or such as an RNA preparation that is enriched for messenger RNA (mRNA), such as RNA preparations that include greater than 70%, or greater than 80%, or greater than 90%, or greater than 95%, or greater than 99% messenger RNA. Typically, RNA
15 preparations that are enriched for messenger RNA are utilized to provide the RNA template in the practice of the methods of this aspect of the invention. Messenger RNA can be purified in accordance with any art-recognized method, such as by the use of oligo-dT columns (*see, e.g.*, Sambrook et al., 1989, *Molecular Cloning - A Laboratory Manual (2nd Ed.)*, Vol. 1, Chapter 7, Cold Spring Harbor Laboratory, Cold Spring
20 Harbor, New York).

Total RNA may be isolated from cells by procedures that involve breaking open the cells and, typically, denaturation of the proteins contained therein. Additional steps may be employed to remove DNA. Cell lysis may be accomplished with a nonionic detergent, followed by microcentrifugation to remove the nuclei and hence the bulk of the
25 cellular DNA. In one embodiment, RNA is extracted from cells using guanidinium thiocyanate lysis followed by CsCl centrifugation to separate the RNA from DNA (Chirgwin et al., 1979, *Biochemistry* 18:5294-5299). Messenger RNA may be selected with oligo-dT cellulose (*see* Sambrook et al., *supra*). Separation of RNA from DNA can also be accomplished by organic extraction, for example, with hot phenol or
30 phenol/chloroform/isoamyl alcohol. If desired, RNase inhibitors may be added to the lysis buffer. Likewise, for certain cell types, it may be desirable to add a protein denaturation/digestion step to the protocol.

The sample of total RNA typically includes a multiplicity of different mRNA molecules, each different mRNA molecule having a different nucleotide sequence (although there may be multiple copies of the same mRNA molecule). In a specific embodiment, the mRNA molecules in the RNA sample comprise at least 100 different
5 nucleotide sequences. In other embodiments, the mRNA molecules of the RNA sample comprise at least 500, 1,000, 5,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000 or 100,000 different nucleotide sequences. In another specific embodiment, the RNA sample is a mammalian RNA sample, the mRNA molecules of the mammalian RNA sample comprising about 20,000 to 30,000 different nucleotide
10 sequences, or comprising substantially all of the different mRNA sequences that are expressed in the cell(s) from which the mRNA was extracted.

In the context of the present example, cDNA molecules are synthesized that are complementary to the RNA template molecules. Each cDNA molecule is preferably sufficiently long (*e.g.*, at least 50 nucleotides in length) to subsequently serve as a
15 specific probe for the mRNA template from which it was synthesized, or to serve as a specific probe for a DNA sequence that is identical to the sequence of the mRNA template from which the cDNA molecule was synthesized. Individual DNA molecules can be complementary to a whole RNA template molecule, or to a portion thereof. Thus, a population of cDNA molecules is synthesized that includes individual DNA molecules
20 that are each complementary to all, or to a portion, of a template RNA molecule. Typically, at least a portion of the complementary sequence of at least 95% (more typically at least 99%) of the template RNA molecules are represented in the population of cDNA molecules.

Any reverse transcriptase molecule can be utilized to synthesize the cDNA
25 molecules, such as reverse transcriptase molecules derived from Moloney murine leukemia virus (MMLV-RT), avian myeloblastosis virus (AMV-RT), bovine leukemia virus (BLV-RT), Rous sarcoma virus (RSV) and human immunodeficiency virus (HIV-RT). A reverse transcriptase lacking RNaseH activity (*e.g.*, SUPERScript II™ sold by Stratagene, La Jolla, California) has the advantage that, in the absence of an RNaseH
30 activity, synthesis of second strand cDNA molecules does not occur during synthesis of first strand cDNA molecules. The reverse transcriptase molecule should also preferably be thermostable so that the cDNA synthesis reaction can be conducted at as high a

temperature as possible, while still permitting hybridization of any required primer(s) to the RNA template molecules.

The synthesis of the cDNA molecules can be primed using any suitable primer, typically an oligonucleotide in the range of ten to 60 bases in length. Oligonucleotides that are useful for priming the synthesis of the cDNA molecules can hybridize to any portion of the RNA template molecules, including the oligo-dT tail. In some embodiments, the synthesis of the cDNA molecules is primed using a mixture of primers, such as a mixture of primers having random nucleotide sequences. Typically, for oligonucleotide molecules less than 100 bases in length, hybridization conditions are 5°C to 10°C below the homoduplex melting temperature (T_m); *see generally*, Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press, 1987; Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing, 1987).

A primer for priming cDNA synthesis can be prepared by any suitable method, such as phosphotriester and phosphodiester methods of synthesis, or automated embodiments thereof. It is also possible to use a primer that has been isolated from a biological source, such as a restriction endonuclease digest. An oligonucleotide primer can be DNA, RNA, chimeric mixtures or derivatives or modified versions thereof, so long as it is still capable of priming the desired reaction. The oligonucleotide primer can be modified at the base moiety, sugar moiety, or phosphate backbone, and may include other appending groups or labels, so long as it is still capable of priming cDNA synthesis.

An oligonucleotide primer for priming cDNA synthesis can be derived by cleavage of a larger nucleic acid fragment using non-specific nucleic acid cleaving chemicals or enzymes or site-specific restriction endonucleases; or by synthesis by standard methods known in the art, *e.g.*, by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.) and standard phosphoramidite chemistry. As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (*Nucl. Acids Res.* 16:3209-3221, 1988), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451).

Once the desired oligonucleotide is synthesized, it is cleaved from the solid support on which it was synthesized and treated, by methods known in the art, to remove any protecting groups present. The oligonucleotide may then be purified by any method

known in the art, including extraction and gel purification. The concentration and purity of the oligonucleotide may be determined, for example, by examining the oligonucleotide that has been separated on an acrylamide gel, or by measuring the optical density at 260 nm in a spectrophotometer.

5 After cDNA synthesis is complete, the RNA template molecules can be hydrolyzed, and all, or substantially all (typically more than 99%), of the primers can be removed. Hydrolysis of the RNA template can be achieved, for example, by alkalization of the solution containing the RNA template (*e.g.*, by addition of an aliquot of a concentrated sodium hydroxide solution). The primers can be removed, for example,
10 by applying the solution containing the RNA template molecules, cDNA molecules, and the primers, to a column that separates nucleic acid molecules on the basis of size. The purified, cDNA molecules, can then, for example, be precipitated and redissolved in a suitable buffer.

 The cDNA molecules are typically labeled to facilitate the detection of the cDNA
15 molecules when they are used as a probe in a hybridization experiment, such as a probe used to screen a DNA microarray, to identify an efficacy-related population of genes. The cDNA molecules can be labeled with any useful label, such as a radioactive atom (*e.g.*, ^{32}P), but typically the cDNA molecules are labeled with a dye. Examples of suitable dyes include fluorophores and chemilumescers.

20 By way of example, cDNA molecules can be coupled to dye molecules via aminoallyl linkages by incorporating allylamine-derivatized nucleotides (*e.g.*, allylamine-dATP, allylamine-dCTP, allylamine-dGTP, and/or allylamine-dTTP) into the cDNA molecules during synthesis of the cDNA molecules. The allylamine-derivatized nucleotide(s) can then be coupled, via an aminoallyl linkage, to N-
25 hydroxysuccinimide ester derivatives (NHS derivatives) of dyes (*e.g.*, Cy-NHS, Cy3-NHS and/or Cy5-NHS). Again by way of example, in another embodiment, dye-labeled nucleotides may be incorporated into the cDNA molecules during synthesis of the cDNA molecules, which labels the cDNA molecules directly.

 It is also possible to include a spacer (usually 5-16 carbon atoms long) between
30 the dye and the nucleotide, which may improve enzymatic incorporation of the modified nucleotides during synthesis of the cDNA molecules.

In the context of the present example, the labeled cDNA is hybridized to a DNA array that includes hundreds, or thousands, of identified nucleic acid molecules (e.g., cDNA molecules) that correspond to genes that are expressed in the type of cells wherein gene expression is being analyzed. Typically, hybridization conditions used to
5 hybridize the labeled cDNA to a DNA array are no more than 25°C to 30°C (for example, 10°C) below the melting temperature (T_m) of the native duplex of the cDNA that has the lowest melting temperature (see generally, Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press, 1987; Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing, 1987). T_m for nucleic acid
10 molecules greater than about 100 bases can be calculated by the formula $T_m = 81.5 + 0.41\%(G+C) - \log(Na^+)$. For oligonucleotide molecules less than 100 bases in length, exemplary hybridization conditions are 5° to 10°C below T_m .

Preparation of microarrays. Nucleic acid molecules can be immobilized on a solid substrate by any art-recognized means. For example, nucleic acid molecules (such
15 as DNA or RNA molecules) can be immobilized to nitrocellulose, or to a synthetic membrane capable of binding nucleic acid molecules, or to a nucleic acid microarray, such as a DNA microarray. A DNA microarray, or chip, is a microscopic array of DNA fragments, such as synthetic oligonucleotides, disposed in a defined pattern on a solid support, wherein they are amenable to analysis by standard hybridization methods
20 (see, Schena, *BioEssays* 18: 427, 1996).

The DNA in a microarray may be derived, for example, from genomic or cDNA libraries, from fully sequenced clones, or from partially sequenced cDNAs known as expressed sequence tags (ESTs). Methods for obtaining such DNA molecules are generally known in the art (see, e.g., Ausubel et al., eds., 1994, *Current Protocols in*
25 *Molecular Biology*, Vol. 2, Current Protocols Publishing, New York). Again by way of example, oligonucleotides may be synthesized by conventional methods, such as the methods described herein.

Microarrays can be made in a number of ways, of which several are described below. However produced, microarrays preferably share certain characteristics. The
30 arrays are preferably reproducible, allowing multiple copies of a given array to be produced and easily compared with each other. Preferably the microarrays are small, usually smaller than 5 cm², and they are made from materials that are stable under nucleic

acid hybridization conditions. A given binding site or unique set of binding sites in the microarray should specifically bind the product of a single gene (or a nucleic acid molecule that represents the product of a single gene, such as a cDNA molecule that is complementary to all, or to part, of an mRNA molecule). Although there may be more
5 than one physical binding site (hereinafter "site") per specific gene product, for the sake of clarity the discussion below will assume that there is a single site.

In one embodiment, the microarray is an array of polynucleotide probes, the array comprising a support with at least one surface and typically at least 100 different polynucleotide probes, each different polynucleotide probe comprising a different
10 nucleotide sequence and being attached to the surface of the support in a different location on the surface. For example, the nucleotide sequence of each of the different polynucleotide probes can be in the range of 40 to 80 nucleotides in length. For example, the nucleotide sequence of each of the different polynucleotide probes can be in the range of 50 to 70 nucleotides in length. For example, the nucleotide sequence of each of the
15 different polynucleotide probes can be in the range of 50 to 60 nucleotides in length. In specific embodiments, the array comprises polynucleotide probes of at least 2,000, 4,000, 10,000, 15,000, 20,000, 50,000, 80,000, or 100,000 different nucleotide sequences.

Thus, the array can include polynucleotide probes for most, or all, genes expressed in a cell, tissue, organ or organism. In a specific embodiment, the cell or
20 organism is a mammalian cell or organism. In another specific embodiment, the cell or organism is a human cell or organism. In specific embodiments, the nucleotide sequences of the different polynucleotide probes of the array are specific for at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% of the genes in the genome of the cell or organism. Most preferably, the nucleotide sequences
25 of the different polynucleotide probes of the array are specific for all of the genes in the genome of the cell or organism. In specific embodiments, the polynucleotide probes of the array hybridize specifically and distinguishably to at least 10,000, to at least 20,000, to at least 50,000, to at least 80,000, or to at least 100,000 different polynucleotide sequences. In other specific embodiments, the polynucleotide probes of the array
30 hybridize specifically and distinguishably to at least 90%, at least 95%, or at least 99% of the genes or gene transcripts of the genome of a cell or organism. Most preferably, the

polynucleotide probes of the array hybridize specifically and distinguishably to the genes or gene transcripts of the entire genome of a cell or organism.

In specific embodiments, the array has at least 100, at least 250, at least 1,000, or at least 2,500 probes per 1 cm², preferably all or at least 25% or 50% of which are different from each other. In another embodiment, the array is a positionally addressable array (in that the sequence of the polynucleotide probe at each position is known). In another embodiment, the nucleotide sequence of each polynucleotide probe in the array is a DNA sequence. In another embodiment, the DNA sequence is a single-stranded DNA sequence. The DNA sequence may be, *e.g.*, a cDNA sequence, or a synthetic sequence.

When a cDNA molecule that corresponds to an mRNA of a cell is made and hybridized to a microarray under suitable hybridization conditions, the level of hybridization to the site in the array corresponding to any particular gene will reflect the prevalence in the cell of mRNA transcribed from that gene. For example, when detectably labeled (*e.g.*, with a fluorophore) DNA complementary to the total cellular mRNA is hybridized to a microarray, the site on the array corresponding to a gene (*i.e.*, capable of specifically binding the product of the gene) that is not transcribed in the cell will have little or no signal (*e.g.*, fluorescent signal), and a gene for which the encoded mRNA is prevalent will have a relatively strong signal.

In some embodiments, cDNA molecule populations prepared from RNA from two different cell populations, or tissues, or organs, or whole organisms, are hybridized to the binding sites of the array. A single array can be used to simultaneously screen more than one cDNA sample. For example, in the context of the present invention, a single array can be used to simultaneously screen a cDNA sample prepared from a living thing that has been contacted with an agent (*e.g.*, candidate partial agonist of PPAR γ), and the same type of living thing that has not been contacted with the agent. The cDNA molecules in the two samples are differently labeled so that they can be distinguished. In one embodiment, for example, cDNA molecules from a cell population treated with a drug is synthesized using a fluorescein-labeled NTP, and cDNA molecules from a control cell population, not treated with the drug, is synthesized using a rhodamine-labeled NTP. When the two populations of cDNA molecules are mixed and hybridized to the DNA array, the relative intensity of signal from each population of cDNA molecules is

determined for each site on the array, and any relative difference in abundance of a particular mRNA detected.

In this representative example, the cDNA molecule population from the drug-treated cells will fluoresce green when the fluorophore is stimulated, and the cDNA molecule population from the untreated cells will fluoresce red. As a result, when the drug treatment has no effect, either directly or indirectly, on the relative abundance of a particular mRNA in a cell, the mRNA will be equally prevalent in treated and untreated cells and red-labeled and green-labeled cDNA molecules will be equally prevalent. When hybridized to the DNA array, the binding site(s) for that species of RNA will emit wavelengths characteristic of both fluorophores (and appear brown in combination). In contrast, when the drug-exposed cell is treated with a drug that, directly or indirectly, increases the prevalence of the mRNA in the cell, the ratio of green to red fluorescence will increase. When the drug decreases the mRNA prevalence, the ratio will decrease.

The use of a two-color fluorescence labeling and detection scheme to define alterations in gene expression has been described, *e.g.*, in Schena et al., 1995, *Science* 270:467-470, which is incorporated by reference in its entirety for all purposes. An advantage of using cDNA molecules labeled with two different fluorophores is that a direct and internally controlled comparison of the mRNA levels corresponding to each arrayed gene in two cell states can be made, and variations due to minor differences in experimental conditions (*e.g.*, hybridization conditions) will not affect subsequent analyses. However, it will be recognized that it is also possible to use cDNA molecules from a single cell, and compare, for example, the absolute amount of a particular mRNA in, *e.g.*, a drug-treated or an untreated cell.

Exemplary microarrays and methods for their manufacture and use are set forth in T.R. Hughes et al., *Nature Biotechnology* 19: 342-347 (April 2001), which publication is incorporated herein by reference.

Preparation of nucleic acid molecules for immobilization on microarrays. As noted above, the "binding site" to which a particular, cognate, nucleic acid molecule specifically hybridizes is usually a nucleic acid, or nucleic acid analogue, attached at that binding site. In one embodiment, the binding sites of the microarray are DNA polynucleotides corresponding to at least a portion of some or all genes in an organism's genome. These DNAs can be obtained by, for example, polymerase chain reaction (PCR)

amplification of gene segments from genomic DNA, cDNA (*e.g.*, by reverse transcription or RT-PCR), or cloned sequences. Nucleic acid amplification primers are chosen, based on the known sequence of the genes or cDNA, that result in amplification of unique fragments (*i.e.*, fragments that typically do not share more than 10 bases of contiguous identical sequence with any other fragment on the microarray). Computer programs are useful in the design of primers with the required specificity and optimal amplification properties. *See, e.g.*, Oligo version 5.0 (National Biosciences). Typically each gene fragment on the microarray will be between about 50 bp and about 2000 bp, more typically between about 100 bp and about 1000 bp, and usually between about 300 bp and about 800 bp in length.

Nucleic acid amplification methods are well known and are described, for example, in Innis et al., eds., 1990, *PCR Protocols: A Guide to Methods and Applications*, Academic Press Inc., San Diego, CA, which is incorporated by reference in its entirety for all purposes. Computer controlled robotic systems are useful for isolating and amplifying nucleic acids.

An alternative means for generating the nucleic acid molecules for the microarray is by synthesis of synthetic polynucleotides or oligonucleotides, *e.g.*, using N-phosphonate or phosphoramidite chemistries (*e.g.*, Froehler et al., 1986, *Nucleic Acid Res* 14:5399-5407). Synthetic sequences are typically between about 15 and about 100 bases in length, such as between about 20 and about 50 bases.

In some embodiments, synthetic nucleic acids include non-natural bases, *e.g.*, inosine. Where the particular base in a given sequence is unknown or is polymorphic, a universal base, such as inosine or 5-nitroindole, may be substituted. Additionally, it is possible to vary the charge on the phosphate backbone of the oligonucleotide, for example, by thiolation or methylation, or even to use a peptide rather than a phosphate backbone. The making of such modifications is within the skill of one trained in the art.

As noted above, nucleic acid analogues may be used as binding sites for hybridization. An example of a suitable nucleic acid analogue is peptide nucleic acid (*see, e.g.*, Egholm et al., 1993, *Nature* 365:566-568; *see also* U.S. Patent No. 5,539,083).

In another embodiment, the binding (hybridization) sites are made from plasmid or phage clones of genes, cDNAs (*e.g.*, expressed sequence tags), or inserts therefrom

(Nguyen et al., 1995, *Genomics* 29:207-209). In yet another embodiment, the polynucleotide of the binding sites is RNA.

Attaching nucleic acids to the solid support. The nucleic acids, or analogues, are attached to a solid support, which may be made, for example, from glass, silicon, plastic (e.g., polypropylene, nylon, polyester), polyacrylamide, nitrocellulose, cellulose acetate or other materials. In general, non-porous supports, and glass in particular, are preferred. The solid support may also be treated in such a way as to enhance binding of oligonucleotides thereto, or to reduce non-specific binding of unwanted substances thereto. For example, a glass support may be treated with polylysine or silane to facilitate attachment of oligonucleotides to the slide.

Methods of immobilizing DNA on the solid support may include direct touch, micropipetting (see, e.g., Yershov et al., *Proc. Natl. Acad. Sci. USA* 93(10):4913-4918 (1996)), or the use of controlled electric fields to direct a given oligonucleotide to a specific spot in the array. Oligonucleotides are typically immobilized at a density of 100 to 10,000 oligonucleotides per cm^2 , such as at a density of about 1000 oligonucleotides per cm^2 .

A preferred method for attaching the nucleic acids to a surface is by printing on glass plates, as is described generally by Schena et al., 1995, *Science* 270:467-470. This method is especially useful for preparing microarrays of cDNA. (See also DeRisi et al., 1996, *Nature Genetics* 14:457-460; Shalon et al., 1996, *Genome Res.* 6:639-645; and Schena et al., *Proc. Natl. Acad. Sci. USA* 93(20):10614-19, 1996.)

In an alternative to immobilizing pre-fabricated oligonucleotides onto a solid support, it is possible to synthesize oligonucleotides directly on the support (see, e.g., Maskos et al., *Nucl. Acids Res.* 21:2269-70, 1993; Lipshutz et al., 1999, *Nat. Genet.* 21(1 Suppl):20-4). Methods of synthesizing oligonucleotides directly on a solid support include photolithography (see McGall et al., *Proc. Natl. Acad. Sci. (USA)* 93:13555-60, 1996) and piezoelectric printing (Lipshutz et al., 1999, *Nat. Genet.* 21(1 Suppl):20-4).

A high-density oligonucleotide array may be employed. Techniques are known for producing arrays containing thousands of oligonucleotides complementary to defined sequences, at defined locations on a surface using photolithographic techniques for synthesis in situ (see, Pease et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:5022-5026; Lockhart et al., 1996, *Nature Biotechnol.* 14:1675-80) or other methods for rapid

synthesis and deposition of defined oligonucleotides (Lipshutz et al., 1999, *Nat. Genet.* 21(1 Suppl):20-4.).

In some embodiments, microarrays are manufactured by means of an ink jet printing device for oligonucleotide synthesis, *e.g.*, using the methods and systems described by Blanchard in International Patent Publication No. WO 98/41531, published September 24, 1998; Blanchard et al., 1996, *Biosensors and Bioelectronics* 11:687-690; Blanchard, 1998, in *Synthetic DNA Arrays in Genetic Engineering*, Vol. 20, J.K. Setlow, Ed., Plenum Press, New York at pages 111-123; U.S. Patent No. 6,028,189 to Blanchard. Specifically, the oligonucleotide probes in such microarrays are preferably synthesized in arrays, *e.g.*, on a glass slide, by serially depositing individual nucleotide bases in "microdroplets" of a high surface tension solvent such as propylene carbonate. The microdroplets have small volumes (*e.g.*, 100 pL or less, more preferably 50 pL or less) and are separated from each other on the microarray (*e.g.*, by hydrophobic domains) to form circular surface tension wells which define the locations of the array elements (*i.e.*, the different probes).

Other methods for making microarrays, *e.g.*, by masking (Maskos and Southern, 1992, *Nuc. Acids Res.* 20:1679-1684), may also be used. In principle, any type of array, for example dot blots on a nylon hybridization membrane (*see* Sambrook et al., 1989, *Molecular Cloning - A Laboratory Manual (2nd Ed.)*, Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), could be used, although, as will be recognized by those of skill in the art, very small arrays are typically preferred because hybridization volumes will be smaller.

Signal detection and data analysis. When fluorescently labeled probes are used, the fluorescence emissions at each site of an array can be detected by scanning confocal laser microscopy. In one embodiment, a separate scan, using the appropriate excitation line, is carried out for each of the two fluorophores used. Alternatively, a laser can be used that allows simultaneous specimen illumination at wavelengths specific to the two fluorophores and emissions from the two fluorophores can be analyzed simultaneously (*see* Shalon et al., 1996, *Genome Research* 6:639-645, which is incorporated by reference in its entirety for all purposes). In one embodiment, the arrays are scanned with a laser fluorescent scanner with a computer controlled X-Y stage and a microscope objective. Sequential excitation of the two fluorophores is achieved with a multi-line, mixed gas

laser and the emitted light is split by wavelength and detected with two photomultiplier tubes. Fluorescence laser scanning devices are described in Shalon et al., 1996, *Genome Res.* 6:639-645 and in other references cited herein. Alternatively, the fiber-optic bundle described by Ferguson et al., 1996, *Nature Biotechnol.* 14:1681-1684, may be used to
5 monitor mRNA abundance levels at a large number of sites simultaneously.

Signals are recorded and may be analyzed by computer, *e.g.*, using a 12 bit analog to digital board. In some embodiments the scanned image is despeckled using a graphics program (*e.g.*, Hijaak Graphics Suite) and then analyzed using an image gridding program that creates a spreadsheet of the average hybridization at each wavelength at
10 each site. If necessary, an experimentally determined correction for "cross talk" (or overlap) between the channels for the two fluorors may be made. For any particular hybridization site on the transcript array, a ratio of the emission of the two fluorophores can be calculated. The ratio is independent of the absolute expression level of the cognate gene, but is useful for genes whose expression is significantly modulated by drug
15 administration.

The relative abundance of an mRNA in two biological samples is scored as a perturbation and its magnitude determined (*i.e.*, the abundance is different in the two sources of mRNA tested), or as not perturbed (*i.e.*, the relative abundance is the same). Preferably, in addition to identifying a perturbation as positive or negative, it is
20 advantageous to determine the magnitude of the perturbation. This can be carried out, as noted above, by calculating the ratio of the emission of the two fluorophores used for differential labeling, or by analogous methods that will be readily apparent to those of skill in the art.

By way of example, two samples, each labeled with a different fluor, are
25 hybridized simultaneously to permit differential expression measurements. If neither sample hybridizes to a given spot in the array, no fluorescence will be seen. If only one hybridizes to a given spot, the color of the resulting fluorescence will correspond to that of the fluor used to label the hybridizing sample (for example, green if the sample was labeled with Cy3, or red, if the sample was labeled with Cy5). If both samples hybridize
30 to the same spot, an intermediate color is produced (for example, yellow if the samples were labeled with fluorescein and rhodamine). Then, applying methods of pattern recognition and data analysis known in the art, it is possible to quantify differences in

gene expression between the samples. Methods of pattern recognition and data analysis are described in *e.g.*, International Publication WO 00/24936, which is incorporated by reference herein.

Measurement of Expression Pattern of an Efficacy-Related Population of

5 Proteins: In the practice of some embodiments of the present invention, the expression pattern of an efficacy-related population of proteins in a living thing is measured. Any useful method for measuring protein expression patterns can be used. Typically all, or substantially all, proteins are extracted from a living thing, or a portion thereof. The living thing is typically treated to disrupt cells, for example by
10 homogenizing the cellular material in a blender, or by grinding (in the presence of acid-washed, siliconized, sand if desired) the cellular material with a mortar and pestle, or by subjecting the cellular material to osmotic stress that lyses the cells. Cell disruption may be carried out in the presence of a buffer that maintains the released contents of the disrupted cells at a desired pH, such as the physiological pH of the cells. The buffer may
15 optionally contain inhibitors of endogenous proteases. Physical disruption of the cells can be conducted in the presence of chemical agents (*e.g.*, detergents) that promote the release of proteins.

The cellular material may be treated in a manner that does not disrupt a significant proportion of cells, but which removes proteins from the surface of the cellular material,
20 and/or from the interstices between cells. For example, cellular material can be soaked in a liquid buffer, or, in the case of plant material, can be subjected to a vacuum, in order to remove proteins located in the intercellular spaces and/or in the plant cell wall. If the cellular material is a microorganism, proteins can be extracted from the microorganism culture medium.

25 It may be desirable to include one or more protease inhibitors in the protein extraction buffer. Representative examples of protease inhibitors include: serine protease inhibitors (such as phenylmethylsulfonyl fluoride (PMSF), benzamide, benzamidinium HCl, ϵ -Amino-*n*-caproic acid and aprotinin (Trasylo1)); cysteine protease inhibitors, such as sodium *p*-hydroxymercuribenzoate; competitive protease inhibitors,
30 such as antipain and leupeptin; covalent protease inhibitors, such as iodoacetate and *N*-ethylmaleimide; aspartate (acidic) protease inhibitors, such as pepstatin and diazoacetylornithine methyl ester (DAN); metalloprotease inhibitors, such as EGTA

[ethylene glycol bis(β -aminoethyl ether) *N,N,N',N'*-tetraacetic acid], and the chelator 1, 10-phenanthroline.

The mixture of released proteins may, or may not, be treated to completely or partially purify some of the proteins for further analysis, and/or to remove non-protein contaminants (*e.g.*, carbohydrates and lipids). In some embodiments, the complete mixture of released proteins is analyzed to determine the amount and/or identity of some or all of the proteins. For example, the protein mixture may be applied to a substrate bearing antibody molecules that specifically bind to one or more proteins in the mixture. The unbound proteins are removed (*e.g.*, washed away with a buffer solution), and the amount of bound protein(s) is measured. Representative techniques for measuring the amount of protein using antibodies are described in Harlow and Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, New York, and include such techniques as the ELISA assay. Moreover, protein microarrays can be used to simultaneously measure the amount of a multiplicity of proteins. A surface of the microarray bears protein binding agents, such as monoclonal antibodies specific to a plurality of protein species. Preferably, antibodies are present for a substantial fraction of the encoded proteins, or at least for those proteins whose amount is to be measured. Methods for making monoclonal antibodies are well known (*see, e.g.*, Harlow and Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, N.Y.). Protein binding agents are not restricted to monoclonal antibodies, and can be, for example, scFv/Fab diabodies, affibodies, and aptamers. Protein microarrays are generally described by M.F. Templin et al., Protein Microarray Technology, *Trends in Biotechnology*, 20(4):160-166(2002). Representative examples of protein microarrays are described by H. Zhu et al., Global Analysis of Protein Activities Using Proteome Chips, *Science*, 293:2102-2105 (2001); and G. MacBeath and S.L. Schreiber, Printing Proteins as Microarrays for High-Throughput Function Determination, *Science*, 289:1760-1763 (2000).

In some embodiments, the released protein is treated to completely or partially purify some of the proteins for further analysis, and/or to remove non-protein contaminants. Any useful purification technique, or combination of techniques, can be used. For example, a solution containing extracted proteins can be treated to selectively precipitate certain proteins, such as by dissolving ammonium sulfate in the solution, or by adding trichloroacetic acid. The precipitated material can be separated from the

unprecipitated material, for example by centrifugation, or by filtration. The precipitated material can be further fractionated if so desired.

By way of example, a number of different neutral or slightly acidic salts have been used to solubilize, precipitate, or fractionate proteins in a differential manner. These include NaCl, Na₂SO₄, MgSO₄ and NH₄(SO₄)₂. Ammonium sulfate is a commonly used precipitant for salting proteins out of solution. The solution to be treated with ammonium sulfate may first be clarified by centrifugation. The solution should be in a buffer at neutral pH unless there is a reason to conduct the precipitation at another pH; in most cases the buffer will have ionic strength close to physiological. Precipitation is usually performed at 0-4°C (to reduce the rate of proteolysis caused by proteases in the solution), and all solutions should be precooled to that temperature range.

Representative examples of other art-recognized techniques for purifying, or partially purifying, proteins from a living thing are exclusion chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, reversed-phase chromatography and immobilized metal affinity chromatography.

Hydrophobic interaction chromatography and reversed-phase chromatography are two separation methods based on the interactions between the hydrophobic moieties of a sample and an insoluble, immobilized hydrophobic group present on the chromatography matrix. In hydrophobic interaction chromatography the matrix is hydrophilic and is substituted with short-chain phenyl or octyl nonpolar groups. The mobile phase is usually an aqueous salt solution. In reversed phase chromatography the matrix is silica that has been substituted with longer *n*-alkyl chains, usually C₈ (octylsilyl) or C₁₈ (octadecylsilyl). The matrix is less polar than the mobile phase. The mobile phase is usually a mixture of water and a less polar organic modifier.

Separations on hydrophobic interaction chromatography matrices are usually done in aqueous salt solutions, which generally are nondenaturing conditions. Samples are loaded onto the matrix in a high-salt buffer and elution is by a descending salt gradient. Separations on reversed-phase media are usually done in mixtures of aqueous and organic solvents, which are often denaturing conditions. In the case of protein purification, hydrophobic interaction chromatography depends on surface hydrophobic groups and is usually carried out under conditions which maintain the integrity of the protein molecule. Reversed-phase chromatography depends on the native hydrophobicity of the protein and

is carried out under conditions which expose nearly all hydrophobic groups to the matrix, *i.e.*, denaturing conditions.

Ion-exchange chromatography is designed specifically for the separation of ionic or ionizable compounds. The stationary phase (column matrix material) carries ionizable functional groups, fixed by chemical bonding to the stationary phase. These fixed charges carry a counterion of opposite sign. This counterion is not fixed and can be displaced. Ion-exchange chromatography is named on the basis of the sign of the displaceable charges. Thus, in anion ion-exchange chromatography the fixed charges are positive and in cation ion-exchange chromatography the fixed charges are negative.

Retention of a molecule on an ion-exchange chromatography column involves an electrostatic interaction between the fixed charges and those of the molecule, binding involves replacement of the nonfixed ions by the molecule. Elution, in turn, involves displacement of the molecule from the fixed charges by a new counterion with a greater affinity for the fixed charges than the molecule, and which then becomes the new, nonfixed ion.

The ability of counterions (salts) to displace molecules bound to fixed charges is a function of the difference in affinities between the fixed charges and the nonfixed charges of both the molecule and the salt. Affinities in turn are affected by several variables, including the magnitude of the net charge of the molecule and the concentration and type of salt used for displacement.

Solid-phase packings used in ion-exchange chromatography include cellulose, dextrans, agarose, and polystyrene. The exchange groups used include DEAE (diethylaminoethyl), a weak base, that will have a net positive charge when ionized and will therefore bind and exchange anions; and CM (carboxymethyl), a weak acid, with a negative charge when ionized that will bind and exchange cations. Another form of weak anion exchanger contains the PEI (polyethyleneimine) functional group. This material, most usually found on thin layer sheets, is useful for binding proteins at pH values above their pI. The polystyrene matrix can be obtained with quaternary ammonium functional groups for strong base anion exchange or with sulfonic acid functional groups for strong acid cation exchange. Intermediate and weak ion-exchange materials are also available. Ion-exchange chromatography need not be performed using a column, and can be

performed as batch ion-exchange chromatography with the slurry of the stationary phase in a vessel such as a beaker.

Gel filtration is performed using porous beads as the chromatographic support. A column constructed from such beads will have two measurable liquid volumes, the external volume, consisting of the liquid between the beads, and the internal volume, consisting of the liquid within the pores of the beads. Large molecules will equilibrate only with the external volume while small molecules will equilibrate with both the external and internal volumes. A mixture of molecules (such as proteins) is applied in a discrete volume or zone at the top of a gel filtration column and allowed to percolate through the column. The large molecules are excluded from the internal volume and therefore emerge first from the column while the smaller molecules, which can access the internal volume, emerge later. The volume of a conventional matrix used for protein purification is typically 30 to 100 times the volume of the sample to be fractionated. The absorbance of the column effluent can be continuously monitored at a desired wavelength using a flow monitor.

A technique that can be applied to the purification of proteins is High Performance Liquid Chromatography (HPLC). HPLC is an advancement in both the operational theory and fabrication of traditional chromatographic systems. HPLC systems for the separation of biological macromolecules vary from the traditional column chromatographic systems in three ways; (1) the column packing materials are of much greater mechanical strength, (2) the particle size of the column packing materials has been decreased 5- to 10-fold to enhance adsorption-desorption kinetics and diminish bandspreading, and (3) the columns are operated at 10-60 times higher mobile-phase velocity. Thus, by way of non-limiting example, HPLC can utilize exclusion chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, reversed-phase chromatography and immobilized metal affinity chromatography.

An exemplary technique that is useful for measuring the amounts of individual proteins in a mixture of proteins is two dimensional gel electrophoresis. This technique typically involves isoelectric focussing of a protein mixture along a first dimension, followed by SDS-PAGE of the focussed proteins along a second dimension (*see, e.g., Hames et al., 1990, Gel Electrophoresis of Proteins: A Practical Approach*, IRL Press,

New York; Shevchenko et al., 1996, *Proc. Nat'l Acad. Sci. U.S.A.* 93:1440-1445; Sagliocco et al., 1996, *Yeast* 12:1519-1533; Lander, 1996, *Science* 274:536-539; and Beaumont et al., *Life Science News*, 7, 2001, Amersham Pharmacia Biotech. The resulting series of protein "spots" on the second dimension SDS-PAGE gel can be measured to reveal the amount of one or more specific proteins in the mixture. The identity of the measured proteins may, or may not, be known; it is only necessary to be able to identify and measure specific protein "spots" on the second dimension gel. Numerous techniques are available to measure the amount of protein in a "spot" on the second dimension gel. For example, the gel can be stained with a reagent that binds to proteins and yields a visible protein "spot" (e.g., Coomassie blue dye, or staining with silver nitrate), and the density of the stained spot can be measured. Again by way of example, all, or most, proteins in a mixture can be measured with a fluorescent reagent before electrophoretic separation, and the amount of fluorescence in some, or all, of the resolved protein "spots" can be measured (see, e.g., Beaumont et al., *Life Science News*, 7, 2001, Amersham Pharmacia Biotech).

Again by way of example, any HPLC technique (e.g., exclusion chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, reversed-phase chromatography and immobilized metal affinity chromatography) can be used to separate proteins in a mixture, and the separated proteins can thereafter be directed to a detector (e.g., spectrophotometer) that detects and measures the amount of individual proteins.

In some embodiments of the invention it is desirable to both identify and measure the amount of specific proteins. A technique that is useful in these embodiments of the invention is mass spectrometry, in particular the techniques of electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), although it is understood that mass spectrometry can be used only to measure the amounts of proteins without also identifying (by function and/or sequence) the proteins. These techniques overcame the problem of generating ions from large, non-volatile, analytes, such as proteins, without significant analyte fragmentation (see, e.g., R. Aebersold and D.R. Goodlett, Mass Spectrometry in Proteomics, *Chemical Reviews*, 102(2): 269-296 (2001)).

Thus, for example, proteins can be extracted from cells of a living thing and individual proteins purified therefrom using, for example, any of the art-recognized

purification techniques described herein (e.g., HPLC). The purified proteins are subjected to enzymatic degradation using a protein-degrading agent (e.g., an enzyme, such as trypsin) that cleaves proteins at specific amino acid sequences. The resulting protein fragments are subjected to mass spectrometry. If the sequence of the complete genome (or at least the sequence of part of the genome) of the living thing from which the proteins were isolated is known, then computer algorithms are available that can compare the observed protein fragments to the protein fragments that are predicted to exist by cleaving the proteins encoded by the genome with the agent used to cleave the extracted proteins. Thus, the identity, and the amount, of the proteins from which the observed fragments are derived can be determined.

Again by way of example, the use of isotope-coded affinity tags in conjunction with mass spectrometry is a technique that is adapted to permit comparison of the identities and amounts of proteins expressed in different samples of the same type of living thing subjected to different treatments (e.g., the same type of living tissue cultured, *in vitro*, in the presence or absence of a candidate drug)(see, e.g., S.P. Gygi et al., Quantitative Analysis of Complex Protein Mixtures Using Isotope-Coded Affinity Tags (ICATs), *Nature Biotechnology*, 17:994-999(1999)). In an exemplary embodiment of this method, two different samples of the same type of living thing are subjected to two different treatments (treatment 1 and treatment 2). Proteins are extracted from the treated living things and are labeled (via cysteine residues) with an ICAT reagent that includes (1) a thiol-specific reactive group, (2) a linker that can include eight deuteriums (yielding a heavy ICAT reagent) or no deuteriums (yielding a light ICAT reagent), and (3) a biotin molecule. Thus, for example, the proteins from treatment 1 may be labeled with the heavy ICAT reagent, and proteins from treatment 2 may be labelled with the light ICAT reagent. The labeled proteins from treatment 1 and treatment 2 are combined and enzymatically cleaved to generate peptide fragments. The tagged (cysteine-containing) fragments are isolated by avidin affinity chromatography (that binds the biotin moiety of the ICAT reagent). The isolated peptides are then separated by mass spectrometry. The quantity and identity of the peptides (and the proteins from which they are derived) may be determined. The method is also applicable to proteins that do not include cysteines by using ICAT reagents that label other amino acids.

Comparison of Gene Expression Levels: Art-recognized statistical techniques can be used to compare the levels of expression of individual genes, or proteins, to identify genes, or proteins, which exhibit significantly different expression levels in treated living things compared to untreated living things, or in diseased living things compared to non-diseased living things. Thus, for example, a t-test can be used to determine whether the mean value of repeated measurements of the level of expression of a particular gene, or protein, is significantly different in a living thing treated with an agent, compared to the same living thing that has not been treated with the agent. Similarly, Analysis of Variance (ANOVA) can be used to compare the mean values of two or more populations (e.g., two or more populations of cultured cells treated with different amounts of a candidate drug) to determine whether the means are significantly different.

The following publications describe examples of art-recognized techniques that can be used to compare the levels of expression of individual genes, or proteins, in treated and untreated living things, or in diseased and non-diseased living things, to identify genes which exhibit significantly different expression levels: *Nature Genetics*, Vol.32, ps. 461-552 (supplement December 2002); *Bioinformatics* 18(4):546-54 (April 2002); Dudoit, et al. *Technical Report 578*, University of California at Berkeley; Tusher et al., *Proc. Nat'l. Acad. Sci. U. S. A.* 98(9):5116-5121 (April 2001); and Kerr, et al., *J. Comput. Biol.* 7: 819-837.

Representative examples of other statistical tests that are useful in the practice of the present invention include the chi squared test which can be used, for example, to test for association between two factors (e.g., transcriptional induction, or repression, by a drug molecule and positive or negative correlation with the presence of a disease state). Again by way of example, art-recognized correlation analysis techniques can be used to test whether a correlation exists between two sets of measurements (e.g., between gene expression and disease state). Standard statistical techniques can be found in statistical texts, such as *Modern Elementary Statistics*, John E. Freund, 7th edition, published by Prentice-Hall; and *Practical Statistics for Environmental and Biological Scientists*, John Townend, published by John Wiley & Sons, Ltd.

Calculation of an Efficacy Value: An efficacy value can be calculated by measuring the response, to an agent, of each individual gene, or protein, within the

efficacy-related population of genes, or efficacy-related population of proteins, to yield a response value for each gene, or protein, within the population, and then performing at least one calculation on all of the response values to yield an efficacy value that numerically represents the expression pattern of the efficacy-related population of genes, or efficacy-related population of proteins, in response to the agent. For example, nucleic acid arrays can be used to measure the response of each individual gene within the efficacy-related gene population, as described *supra*. Again by way of example, Northern blots may be used to measure the response of each individual gene within the efficacy-related gene population. Measurement of gene expression is usually easier *in vitro* than *in vivo*, and an *in vitro* system is usually better adapted to facilitate high-throughput screening of multiple agents.

An efficacy value can be calculated by any suitable means. For example, a living thing (*e.g.*, a rat heart) is contacted with a reference agent (possessing a known biological activity) in a multiplicity of identical, separate, experiments, and the level of expression of each individual gene, or protein, within an efficacy-related gene or protein population, in response to the reference agent, is measured in each of the multiplicity of experiments. The average expression value for each of the genes, or proteins, is calculated by adding together the expression values from each of the multiplicity of experiments, and dividing the sum by the number of experiments.

The same type of living thing (*e.g.*, a rat heart) is contacted with a candidate agent in a multiplicity of identical, separate, experiments, and the level of expression of each individual gene, or protein, within an efficacy-related gene or protein population, in response to the candidate agent, is measured in each of the multiplicity of experiments. The average expression value for each of the genes, or proteins, is calculated by adding together the expression values from each of the multiplicity of experiments, and dividing the sum by the number of experiments.

The average expression value for each gene in response to the candidate agent is divided by the average expression value for each gene in response to the reference agent to yield a percentage expression value for each gene. The mean of all of the percentage expression values is calculated and is the efficacy value for the candidate agent. Similarly, if protein expression levels are being measured, the average expression value for each protein in response to the candidate agent is divided by the average expression

value for each protein in response to the reference agent to yield a percentage expression value for each protein. The mean of all of the percentage expression values is calculated and is the efficacy value for the candidate agent.

By way of further example, the log(ratio)s of the expression levels of all of the genes, or proteins, within an efficacy-related population can be represented by a single scale factor (which is the efficacy value for the agent that caused the gene expression pattern or the protein expression pattern). Exemplary methods for calculating the scale factor S include:

$$(1). S = \sum_{i=1}^n X_i / \sum_{i=1}^n R_i \quad ; n \text{ stands for the number of genes and/or proteins.}$$

$$(2). S = (\sum_{i=1}^n X_i / R_i) / n$$

$$(3). \text{Fit a straight line by: } X_i = S * R_i$$

$$(4). \text{Least } \chi^2 \text{ fitting: choose a value of } S \text{ to minimize the } \chi^2 :$$

$$\chi^2 = \sum_{i=1}^n (S * R_i - X_i)^2 / (\sigma_{R_i}^2 + \sigma_{X_i}^2)$$

$$(5). \text{Least square fitting: choose a value of } S \text{ to minimize the } Q^2 :$$

$$Q^2 = \sum_{i=1}^n (S * R_i - X_i)^2$$

In the foregoing formulae, R_i , σ_{R_i} stand for the log(Ratio) and error of the log(Ratio) for i th gene, or i th protein, from the template experiment, X_i and σ_{X_i} stand for the log(Ratio) and error of log(Ratio) of the same gene, or protein, expressed in response to a candidate agent. The template experiment is the experiment that yields gene expression data, or protein expression data, in response to an agent having a known biological activity. For example, in the context of using the methods of the invention to identify new agonists of PPAR γ , the template experiment is treatment of a living thing with at least one known agonist of PPAR γ to yield an efficacy-related gene expression pattern, and/or protein expression pattern, that is characteristic of the known agonist of PPAR γ .

Use of a Scale of Efficacy Values: In some embodiments of the methods of this aspect of the invention, an efficacy value of an agent is compared to a scale of efficacy values, typically a continuous scale of efficacy values. The scale of efficacy values can

be constructed, for example, by calculating an efficacy value for a reference agent that is known to stimulate a target biological response. This efficacy value forms the upper limit of a continuous scale of efficacy values. The lower limit of the scale can be any value that is less than the efficacy value that forms the upper limit of the scale. For example, the lower limit of the continuous scale can be zero, and the upper limit of the continuous scale can be 1.0. If desired, the scale can be divided into a number of spaced divisions, usually equally spaced divisions, thereby facilitating comparison of an efficacy value of an agent to the scale. For example, a scale that extends from a value of 0 to a value of 1.0 can be divided into the following equally spaced divisions: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0. Optionally, efficacy values can be generated for a multiplicity of reference agents (*e.g.*, 10, 20, 30, 40 or 50 reference agents) that each stimulate the same target, biological, response to different degrees, thereby generating a scale of efficacy values wherein each of the values are actually calculated from expression patterns of an efficacy-related gene population and/or an efficacy-related protein population.

Thus, for example, the upper limit of a continuous scale of efficacy values can be a value of 1.0, which is the efficacy value of a reference agent that is known to stimulate a target biological response. The lower limit of the scale can be arbitrarily set as zero. If the efficacy value of a candidate agent is 0.9, then it can be inferred that the candidate agent is also likely to stimulate the target biological response, because the efficacy value of the candidate agent is close to the efficacy value of the reference agent that is known to stimulate the target biological response.

Toxicity Values and Toxicity-Related Populations of Genes and Proteins: The methods of the invention, for determining whether an agent possesses a defined biological activity, can include the step of comparing a toxicity value of an agent to at least one reference toxicity value to yield a toxicity comparison result, wherein each toxicity value represents at least one expression pattern of the same toxicity-related population of genes or toxicity-related population of proteins. In some embodiments, a toxicity value of the agent is compared to a scale of toxicity values to yield a toxicity comparison result, wherein each toxicity value represents at least one expression pattern of the same toxicity-related population of genes or toxicity-related population of proteins.

A toxicity value is a value that numerically represents the level of expression, in response to an agent, of one of the following: (1) all of the genes within a toxicity-related

population of genes; or (2) all of the proteins within a toxicity-related population of proteins. The toxicity-related population of genes, or the toxicity-related population of proteins, yields at least one expression pattern, in response to an agent, that correlates (positively or negatively) with the presence of at least one undesirable biological response caused by the agent in a living thing.

The gene expression pattern of a toxicity-related population of genes, or proteins, induced by an agent, and, therefore, the toxicity value calculated from the induced gene expression pattern, or protein expression pattern, provides an indication of the extent to which an agent induces one or more undesirable effect(s) in a living thing. Thus, the ability of an agent to induce one, or more, undesirable effect(s) in a living thing can be compared to the ability of one or more other agents to induce the same undesirable effect(s) in the same living thing.

It is typically easier, and more readily informative, to compare toxicity values for different agents, than to directly compare the gene expression patterns, or protein expression patterns, induced in a toxicity-related population of genes or proteins by the agents. For example, comparison of toxicity values can be used to determine whether a candidate inhibitor of a target biological response (*e.g.*, a candidate inhibitor of cholesterol synthesis in the mammalian liver) causes the same undesirable biological effects (*e.g.*, destruction of liver cells) as a known inhibitor of the same target biological response. Thus, the toxicity value of the candidate inhibitor of the target biological response is compared to the toxicity value of the known inhibitor of the same target, biological, response to determine whether the two toxicity values are similar. If the toxicity value of the known inhibitor is similar to the toxicity value of the candidate inhibitor, then it is inferred that the candidate inhibitor causes the same, or similar, undesirable biological responses as the known inhibitor.

Again by way of example, in the context of comparing candidate inhibitors of a target biological response to determine which candidate inhibitor is also the weakest inducer of a specific, undesirable, side-effect, the toxicity values of each candidate inhibitor are compared to each other, and it is inferred that the candidate inhibitor that has the numerically smallest toxicity value is the weakest inducer of the undesirable side-effect.

By way of further example, comparison of toxicity values can be used to identify a partial agonist of a specific biological response (*e.g.*, reduction in the amount of glucose in the blood plasma of a diabetic human being). Typically, an agonist of a target biological response elicits more additional biological responses, including undesirable responses, than a partial agonist of the same target biological response. Consequently, partial agonists of a target biological response are usually preferred over agonists of the target biological response for use as therapeutic agents for treating diseases in which the target biological response is malfunctioning. Thus, when screening candidate therapeutic agents that affect the target biological response, it may be desirable to know whether a candidate agent acts more like a known agonist of the target biological response (and so may have more adverse side effects), or whether the candidate agent acts more like a known partial agonist of the target biological response (and so may have fewer adverse side effects). To this end, a population of genes, or proteins, is identified that yields an expression pattern that correlates (positively or negatively) with the induction of one or more undesirable effects in a living thing in response to a known agonist of the target biological response, and that also yields a different expression pattern that correlates (positively or negatively) with the induction of one or more undesirable effects in the same living thing in response to the partial agonist. This is the population of toxicity-related genes or the population of toxicity-related proteins. Typically, the population of toxicity-related genes, or the population of toxicity-related proteins, is the population of toxicity-related genes, or the population of toxicity-related proteins, that yields expression patterns that most clearly distinguish between the agonist and the partial agonist.

A toxicity value is calculated for the agonist, and a toxicity value is calculated for the partial agonist. A toxicity value is also calculated for the candidate agent, and this value is compared to the toxicity value calculated for the agonist, and to the toxicity value calculated for the partial agonist. The result of this comparison reveals whether the gene or protein expression pattern induced by the candidate agent is more like the gene or protein expression pattern induced by the agonist, or is more like the gene or protein expression pattern induced by the partial agonist. In this example, the candidate agent would be selected for further study if its toxicity value is closer to the toxicity value of the known partial agonist than to the toxicity value of the known agonist.

A toxicity-related population of genes or proteins may be identified, for example, by contacting a living thing (e.g., living tissue, living organ or living organism), or population of living things (e.g., population of living cells in culture), with an agent that is known to cause at least one undesirable biological response that is to be measured using the toxicity-related population of genes or proteins. A population of genes or proteins is identified in the living thing that yields at least one expression pattern that correlates (positively or negatively) with the occurrence of the undesirable biological response(s) caused by the agent. This is the toxicity-related population of genes or proteins. The techniques used to measure and analyze gene expression, or protein expression (e.g., gene expression analysis using DNA microarrays, protein expression analysis using protein microarrays) to identify a toxicity-related population of genes or proteins are the same as the techniques that are useful for measuring and analyzing gene expression or protein expression to identify an efficacy-related population of genes or proteins, as described *supra*.

Example 2 herein describes the identification of toxicity-related populations of genes that are useful for determining whether the undesirable effects induced by a candidate agent in a living thing are more like the undesirable effects induced in the same living thing by a known agonist of PPAR γ , or are more like the undesirable effects induced in the same living thing by a known partial agonist of PPAR γ .

In some embodiments of the methods of the invention, the toxicity-related population of genes or proteins yields at least one toxicity-related gene expression pattern, in response to an agent, that correlates (positively or negatively) with the presence of at least one undesirable biological response caused by the agent in a living thing, wherein the at least one toxicity-related gene expression pattern, or toxicity-related protein expression pattern, appears before the undesirable biological response. Thus, for example, these embodiments of the methods of the invention are particularly useful for high-throughput screening of numerous drug candidates because it is not necessary to wait for the appearance of the undesirable biological response in order to identify those drug candidates that cause the undesirable biological response.

Calculation of Toxicity Values: A toxicity value is calculated by measuring the response, to an agent, of each individual gene or protein within the toxicity-related gene population, or toxicity-related protein population, to yield a response value for each gene

or protein within the population, and then performing at least one calculation on all of the response values to yield a toxicity value that numerically represents the expression pattern of the toxicity-related population of genes, or toxicity-related protein population, in response to the agent. A toxicity value can be calculated by any suitable method, such as the exemplary methods described, *supra*, for calculating an efficacy value.

Use of a Scale of Toxicity Values: In some embodiments of the methods of this aspect of the invention, a toxicity value of an agent is compared to a scale of toxicity values, typically a continuous scale of toxicity values. The scale of toxicity values can be constructed, and used, with the same techniques useful for constructing and using a scale of efficacy values. For example, a scale of toxicity values can be constructed by calculating a toxicity value for a reference agent that is known to stimulate an undesirable biological response. This toxicity value forms the upper limit of a continuous scale of toxicity values. The lower limit of the scale can be any value that is less than the toxicity value that forms the upper limit of the scale. For example, the lower limit of the continuous scale can be zero, and the upper limit of the continuous scale can be 1.0. Thus, for example, if the toxicity value of a candidate agent is 0.9, then it can be inferred that the candidate agent is likely to stimulate the undesirable biological response, because the toxicity value of the candidate agent is close to the toxicity value of the reference agent that is known to stimulate the undesirable biological response.

Classifier Values: The methods of this aspect of the invention can include the step of comparing a classifier value of an agent to at least one reference classifier value to yield a classifier comparison result, wherein each classifier value represents at least one expression pattern of the same classifier population of genes, or classifier population of proteins. In some embodiments, a classifier value of the agent is compared to a scale of classifier values to yield a classifier comparison result, wherein each classifier value represents at least one expression pattern of the same classifier population of genes, or classifier population of proteins.

A classifier value numerically represents the level of expression, in response to an agent, of one of the following: (1) all of the genes within a classifier population of genes; or (2) all of the proteins within a classifier population of proteins. A classifier population of genes or proteins yields different gene expression patterns, or protein expression patterns, and different calculated classifier values, in response to different reference

agents that have different biological activities (*e.g.*, an agonist and a partial agonist of the same target biological response). The gene expression pattern, or protein expression pattern, induced by an agent in the classifier population of genes or proteins correlates (positively or negatively) with the occurrence of the biological activity of the agent.

5 Thus, the biological activities of different agents can be grouped into one, or more, classes based on the gene expression pattern, or protein expression pattern, induced by an agent in one, or more, classifier population(s) of genes or proteins. It is typically easier, and more readily informative, to compare classifier values for different agents, than to compare the gene expression patterns from which the classifier values are calculated.

10 Thus, for example, the classifier value of a candidate agent (*e.g.*, a candidate therapeutic drug molecule) can be compared to the classifier value of a first reference agent that possesses a known biological activity, and to the classifier value of a second reference agent, that possesses a known biological activity that is different from the biological activity of the first reference agent. The comparison reveals whether the gene
15 expression pattern, or protein expression pattern, induced by the candidate agent (and, by implication, the biological activity of the candidate agent) is more like the gene expression pattern, or protein expression pattern, induced by the first reference agent, or is more like the gene expression pattern, or protein expression pattern, induced by the second reference agent. The biological activity of the candidate agent can thereby be
20 classified as being more like the first reference agent, or as being more like the second reference agent.

By way of specific example, the first reference agent may be an agonist of a target biological response in a living thing, and the second reference agent may be a partial agonist of the same target biological response in the same living thing. The agonist
25 stimulates the target biological response in the living thing, but also stimulates other biological responses which may be toxic, or otherwise undesirable, to the living thing. The partial agonist stimulates the same target biological response as the agonist, but stimulates fewer, potentially undesirable, biological responses compared to the agonist. Thus, an agonist is likely to have more undesirable side effects than a partial agonist.

30 To determine whether a candidate agent has a biological activity that is more like the biological activity of an agonist of a specific biological response, or is more like the biological activity of a partial agonist of the same biological response, a living thing is

contacted with the candidate agent, and the expression pattern of a classifier population of genes, or the expression pattern of a classifier population of proteins, in the living thing is measured. The classifier population of genes, or classifier population of proteins, yields a different expression pattern, and, hence, a different calculated classifier value, in response to the agonist than in response to the partial agonist. A classifier value is calculated for the agonist, and a classifier value is calculated for the partial agonist. A classifier value is also calculated for the candidate agent, and this value is compared to the classifier value calculated for the agonist, and to the classifier value calculated for the partial agonist. The result of this comparison reveals whether the gene expression pattern, or protein expression pattern, induced by the candidate agent is more like the gene expression pattern, or protein expression pattern, induced by the agonist, or is more like the gene expression pattern, or protein expression pattern, induced by the partial agonist.

A classifier population of genes, or classifier population of proteins, can be identified, for example, by contacting a living thing (*e.g.*, living tissue, living organ or living organism), or population of living things (*e.g.*, population of living cells in culture), with an agent that is known to cause a target biological response. A population of genes, or a population of proteins, is identified in the living thing that yields at least one expression pattern that correlates (positively or negatively) with the occurrence of the target biological response caused by the agent. The foregoing procedure is repeated with a second reference agent, possessing a different biological activity than the first reference agent, to yield a gene expression pattern, or a protein expression pattern, that is characteristic of the second reference agent. The gene expression pattern, or protein expression pattern, of the first reference agent, and the gene expression pattern, or protein expression pattern, of the second reference agent, are compared to identify the population of genes, or proteins (within the total population of genes, or proteins, whose expression is affected by either the first or second reference agents) that produces an expression pattern that most clearly distinguishes between the first reference agent and the second reference agent. This population of genes, or proteins, is the classifier population. It is understood that the same general method can be used to identify a classifier population of genes, or a classifier population of proteins, that distinguishes between two or more reference agents.

Classifier populations of genes can be identified, for example, in the following manner. Living cells are contacted, *in vivo* or *in vitro*, with an amount of a first reference agent that maximally induces (or maximally inhibits) a target biological response. Messenger RNA is extracted from the contacted cells and used as a template to synthesize cDNA which is then labeled (*e.g.*, with a fluorescent dye). The labeled cDNA is used to probe a DNA array that includes hundreds, or thousands, of identified nucleic acid molecules (*e.g.*, cDNA molecules) that correspond to genes that are expressed in the type of cells that were contacted with the first reference agent. The labeled cDNA molecules that hybridize to the nucleic acid molecules immobilized on the DNA array are identified, and the level of expression of each hybridizing cDNA is measured and compared to the level of expression of the same mRNA molecules in a control sample from living cells that were not contacted with the first reference agent, to yield a gene expression pattern that is induced by the first reference agent.

The foregoing procedure is repeated with a second reference agent, possessing a different biological activity compared to the first reference agent, to yield a gene expression pattern that is characteristic of the second reference agent. For example, the first reference agent may be an agonist of a biological response, and the second reference agent may be a partial agonist of the same biological response. The gene expression pattern of the first reference agent, and the gene expression pattern of the second reference agent, are compared to identify the population of genes (within the total population of genes whose expression is affected by either the first or second reference agents) that produces an expression pattern that most clearly distinguishes between the first reference agent and the second reference agent. This population of genes is the classifier population. In the context of the present example, the classifier population permits classification of a candidate agent as being more similar to the first reference agent than to the second reference agent, or as being more similar to the second reference agent than to the first reference agent. Example 3 herein describes the identification of a classifier population of genes that is useful for classifying candidate agents as being more like an agonist of PPAR γ , or as being more like a partial agonist of PPAR γ .

Classifier populations of proteins can be identified, for example, using the same foregoing approach for identifying classifier populations of genes, except that techniques for measuring the amount of individual proteins (*e.g.*, two dimensional gel

electrophoresis) are used instead of techniques for measuring the amount of individual genes.

5 Calculating a Classifier Value: A classifier value is calculated by measuring the response, to an agent, of each individual gene, or protein, within the classifier gene population, or within the classifier protein population, to yield a response value for each gene within the population, or each protein within the population, and then performing a calculation on all of the response values to yield a classifier value that numerically represents the expression pattern of the classifier population of genes, or proteins, in response to the agent. A classifier value can be calculated by any suitable method, such as the exemplary methods described, *supra*, for calculating an efficacy value.

10 Use of a Scale of Classifier Values: In some embodiments of the methods of this aspect of the invention, a classifier value of an agent is compared to a scale of classifier values, typically a continuous scale of classifier values. The scale of classifier values can be constructed, and used, with the same techniques useful for constructing and using a scale of efficacy values or toxicity values. For example, a scale of classifier values can be constructed by generating classifier values for two reference agents. For example, the classifier value for a partial agonist of a biological response may be 0.1, and the classifier value for an agonist of the same biological response may be 1.0. Thus, the scale of classifier values extends from 0.1 (the classifier value that is most characteristic of a partial agonist of the biological response), to 1.0 (the classifier value that is most characteristic of an agonist of the biological response). Thus, for example, the classifier value of a candidate agent may be 0.6, which is closer to the classifier value of the agonist (1.0), than to the classifier value of the partial agonist (0.1), suggesting that the candidate agent is more likely to be an agonist of the target biological response than a partial agonist of the target biological response.

20 Practicing the methods of the invention *in vitro*: In some embodiments of the methods of the invention, the expression pattern of one, or more, of the classifier population of genes (or classifier population of proteins), the toxicity-related population of genes (or toxicity-related population of proteins), and the efficacy-related population of genes (or efficacy-related population of proteins) is/are measured in the same population of living cells cultured *in vitro*. The use of a population of living cells, cultured *in vitro*, to measure gene expression patterns, or protein expression patterns,

facilitates rapid, high throughput, screening of numerous agents. Representative examples of living cells that can be cultured *in vitro* and used in the practice of the present invention to measure the expression pattern of one, or more, of the classifier population of genes (or classifier population of proteins), the toxicity-related population of genes (or toxicity-related population of proteins), and the efficacy-related population of genes (or efficacy-related population of proteins), are 3T3L1 adipocyte cells (available from the American Type Culture Collection, Manassas, Virginia, as cell line CL-173), hepatocyte cells, myocardiocyte cells, human primary hepatocytes and HEPG2 cells (available from the American Type Culture Collection, Manassas, Virginia, as cell line HB-8065).

Typically, but not necessarily, cultured cells are chosen that correspond to the cells that are affected, *in vivo*, by the agent(s) whose biological activity will be assessed using the cultured cells. For example, cultured liver cells may be used in the practice of the methods of the invention to screen candidate chemical agents that affect an aspect of liver metabolism (*e.g.*, cholesterol synthesis). Similarly, cultured myocardiocyte cells may be used in the practice of the methods of the invention to screen candidate chemical agents that affect an aspect of heart cell metabolism, or cardiac function. Again by way of example, cultured human myoblasts may be used to identify agents that possess the undesirable property of causing cardiac myopathy.

In some embodiments of the methods of the invention, the expression pattern of at least one member of the group consisting of the classifier population of genes (or classifier population of proteins), the toxicity-related population of genes (or toxicity-related population of proteins), and the efficacy-related population of genes (or efficacy-related population of proteins) is measured *in vivo*, and the expression pattern of at least one of the foregoing populations of genes or proteins is measured *in vitro*. For example, chemical agents that affect an aspect of cardiac function (*e.g.*, reduce heart size in a human subject suffering from cardiomyopathy) may be identified by measuring the expression of an efficacy-related gene population in heart tissue of experimental animals treated with candidate agents. Undesirable adverse effects of the candidate agents can be identified by measuring the expression of a toxicity-related gene population in a cardiomyocyte cell population cultured *in vitro*.

In some embodiments, the expression pattern of a toxicity-related population of genes (or toxicity-related population of proteins), and/or the expression pattern of an efficacy-related population of genes (or efficacy-related population of proteins) is/are measured, *in vitro*, using cultured cells that are different from the type(s) of cells that are predominantly (or exclusively) affected, *in vivo*, by the agent(s) whose biological activity will be assessed using the cultured cells. In these embodiments, the living cells that are used to measure the expression pattern of the toxicity-related population of genes (or toxicity-related population of proteins), and/or the expression pattern of the efficacy-related population of genes (or efficacy-related population of proteins), are typically easier to culture and assay than the cells that suffer the undesirable biological effect(s), or exhibit the desired biological effect(s), *in vivo*.

For example, one type of undesirable effect caused by some therapeutic molecules (*e.g.*, rosiglitazone) administered to mammalian subjects is enlargement of the heart, which may also be accompanied by an increase in blood plasma volume. One way to measure these types of undesirable effects is to measure the gene expression pattern of a toxicity-related population of genes in heart tissue of experimental animals (*e.g.*, rats) treated with agents that cause these effects. In some embodiments of the methods of the present invention, however, a more convenient way to measure these changes is to identify cells or tissue that are culturable *in vitro*, and that exhibit changes in gene expression that correlate with, and preferably precede, the changes in heart size and/or plasma volume observed *in vivo*. An example of culturable mammalian cells that meet the foregoing criteria with respect to changes in gene expression are mouse 3T3L1 adipocyte cells.

As described in Example 2, in one option for using 3T3L1 adipocyte mouse cells in the practice of the invention, one, or more, of a classifier population of genes, a toxicity-related population of genes, and an efficacy-related population of genes is/are identified in rat epididymal white adipose tissue (EWAT), *in vivo*, in accordance with the teachings of the present patent application. Thereafter, the classifier population of genes, and/or the toxicity-related population of genes, and/or the efficacy-related population of genes is/are mapped onto 3T3L1 mouse adipocytes.

Use of the classifier comparison result, and/or toxicity comparison result, and/or efficacy comparison result to determine whether an agent possesses a defined biological

activity: In the practice of the methods of the present invention, one or more of the classifier comparison result, the toxicity comparison result, and/or the efficacy comparison result is/are used to determine whether an agent possesses a defined biological activity. For example, any one of the classifier comparison result, the toxicity comparison result, or the efficacy comparison result may be used alone to determine whether an agent possesses a defined biological activity. More typically, one of the following combinations of comparison results is used to determine whether an agent possesses a defined biological activity: efficacy comparison result and toxicity comparison result; efficacy comparison result and classifier comparison result; classifier comparison result and toxicity comparison result; toxicity comparison result and efficacy comparison result and classifier comparison result.

The choice of which comparison result, or combination of comparison results, to use to determine whether an agent possesses a defined biological activity, and the weight to give each comparison result when a combination of comparison results is used, mainly depends on the type and magnitude of the defined biological activity that candidate agents desirably possess. The precise weight to give to a comparison result is a decision that is made in the context of a particular experiment, and is a matter of judgment. For example, an investigator might identify a population of chemical compounds that are potent stimulants of a target biological process, and are therefore candidate therapeutic agents for treating diseased subjects in which the target biological process is inactive, or active at a low level, thereby causing disease. The investigator may want to identify those compounds within the population that cause the least number of undesirable side effects. Thus, for example, the investigator may use only the toxicity comparison result to select candidate therapeutic agents (that cause the least number of undesirable side effects) from among the population of chemical compounds that stimulate the target biological response. If the investigator uses one or more comparison results in addition to the toxicity comparison result, such as the combination of the toxicity comparison result and the efficacy comparison result, the investigator may give most weight to the toxicity comparison result since, in this example, all of the compounds are about equally effective stimulants of the target biological process, and the investigator is most interested in identifying those compounds that cause fewest adverse side-effects.

Again by way of example, an investigator might want to identify a chemical compound that is a potent stimulant of a target biological response, but which does not induce a defined, undesirable, side effect. Thus, the investigator may use the combination of an efficacy comparison result and a toxicity comparison result to
5 determine whether an agent is a potent stimulant of the target biological response, but does not induce the undesirable side effect. Since, in this example, the investigator considers the ability of a compound to stimulate the target biological response to be about equally important as the inability of the compound to induce the undesirable side effect, the investigator may give equal weight, or approximately equal weight, to the efficacy
10 comparison result and to the toxicity comparison result.

The use of other comparison results, in addition to an efficacy comparison result, and/or a toxicity comparison result, and/or a classifier comparison result, is also within the scope of the invention. Thus, using the techniques described herein, a comparison result can be obtained for any measurable biological response. For example, agonists and
15 partial agonists of PPAR γ receptors may also stimulate a related class of molecules called PPAR α receptors. Thus, using the techniques described herein, a population of genes, or proteins, can be identified that yield an expression pattern that correlates (positively or negatively) with the stimulation of PPAR α receptors by an agent. This population of genes, or proteins, can be used to screen candidate PPAR γ agonists, or partial agonists, to
20 identify those candidate agents that possess the undesirable property of stimulating PPAR α receptors.

In another aspect, the present invention provides populations of nucleic acid molecules that are useful in the practice of the methods of the present invention as probes for measuring the level of expression of members of a classifier population of genes, or
25 an efficacy-related population of genes, or a toxicity-related population of genes, wherein the classifier population of genes, the efficacy-related population of genes, and the toxicity-related population of genes are each useful for identifying agonists, or partial agonists, of PPAR γ .

In a further aspect, the present invention provides populations of oligonucleotide
30 probes and populations of genes. The populations of genes include classifier populations of genes, efficacy-related populations of genes, and toxicity-related populations of genes, and are useful, for example, for determining whether an agent possesses a defined

biological activity in accordance with the teachings of the present patent application. The populations of oligonucleotide probes are useful, for example, for measuring the expression patterns of classifier populations of genes, efficacy-related populations of genes, or toxicity-related populations of genes of the present invention.

5 For example, as more fully described in Example 1 herein, Table 1, entitled "PPARg_Mouse_Efficacy_Probe_52 (Species: db/db Mouse)", sets forth an efficacy-related population of mouse genes (SEQ ID NOs: 1-50). The population of 52 oligonucleotide probes identified in Table 1 (SEQ ID NOs: 51-102), and the population of 22 oligonucleotide probes (SEQ ID NOs: 52, 53, 58, 59, 65, 66, 68, 69, 71, 73, 75, 76,
10 78, 82, 86, 88-90, 93, 94, 96, 101) identified in Table 2, entitled "PPARg_3T3L1_Efficacy_Probe_22 (Species: Mouse Cell Line)", are useful in the practice of the methods of the invention to measure the expression pattern of some or all of the efficacy-related population of genes (SEQ ID NOs: 1-50) described in Table 1.

Again by way of example, as more fully described in Example 2 herein, Table 4
15 sets forth a rat toxicity-related population of genes (SEQ ID NOs: 103-152), and a population of oligonucleotide probes (SEQ ID NOs: 153-207) that are useful in the practice of the present invention to measure the expression pattern of the toxicity-related population of genes (SEQ ID NOs: 103-152). Again by way of example, Table 5 sets forth a toxicity-related population of 5 mouse genes (SEQ ID NOs: 208-212) that are
20 useful as early reporters of heart toxicity. Table 5 sets forth a population of oligonucleotide probes (SEQ ID NOs: 213-218) that are useful for measuring the expression pattern of the toxicity-related population of 5 genes (SEQ ID NOs: 208-212).

Again by way of example, Table 6 sets forth a rat toxicity-related population of genes (SEQ ID NOs: 219-550, 104, 105, 112, 119, 126, 127, 133, 136, 149, 150 and 151),
25 and a population of oligonucleotide probes (SEQ ID NOs: 551-894, 155, 157, 164, 171, 178, 179, 185, 188, 204, 205, and 206) that are useful in the practice of the present invention to measure the expression pattern of the toxicity-related populations of genes (SEQ ID NOs: 219-550, 104, 105, 112, 119, 126, 127, 133, 136, 149, 150 and 151).

Table 7 sets forth a mouse cell line toxicity-related population of genes (SEQ ID
30 NOs: 895-949, 42 and 45), and a population of oligonucleotide probes (SEQ ID NOs: 950-1019, 863, 93, 94, and 97) that are useful in the practice of the present invention to

measure the expression pattern of the toxicity-related populations of genes (SEQ ID NOs: 895-949, 42 and 45).

Table 8 sets forth a mouse tissue toxicity-related population of genes (SEQ ID NOs: 1020-1035, 896, 900, 902, 903, 905, 906, 13, 908, 912, 917-920, 925, 926, 929, 932, 934, 936-938, 42, 939, 942, 45, 943-946 and 949), and a population of oligonucleotide probes (SEQ ID NOs: 1036-1057, 951, 955, 957, 863, 959, 960, 63, 962, 966, 971-974, 980, 981, 984, 987, 989, 991-996, 93, 998, 94, 999-1001, 1004, 97, 1005-1014, and 1017-1019) that are useful in the practice of the present invention to measure the expression pattern of the toxicity-related populations of genes (SEQ ID NOs: 1020-1035, 896, 900, 902, 903, 905, 906, 13, 908, 912, 917-920, 925, 926, 929, 932, 936-938, 42, 939, 942, 45, 943-946 and 949).

Table 9 sets forth a rat tissue toxicity-related population of genes (SEQ ID NOs: 1058-1238, 222, 224, 106, 226, 235, 237, 239, 246, 253, 258, 261, 270, 273, 274, 278, 111, 286, 302-304, 307, 308, 316-318, 322, 327, 119, 342, 358, 361, 367-368, 373, 381, 388, 401, 406, 409-410, 416-418, 423, 427-428, 430-432, 434, 439, 441, 447, 450, 455, 461, 464-465, 136, 137, 139, 474, 475, 482, 485, 488, 491, 492, 496, 500, 504, 524, 530, 534, 536, 541, 542, and 547), and a population of oligonucleotide probes (SEQ ID NOs: 1239-1428, 558, 561, 158, 565, 574, 576, 578, 585, 592, 597, 600, 609, 612, 613, 617, 163, 625, 641-643, 646, 647, 655-657, 661, 666, 171, 681, 697, 700, 706, 707, 712, 720, 727, 740, 745, 748, 749, 755-757, 762, 766-767, 769-771, 773, 778, 780, 786, 789, 794, 800, 803-804, 188-189, 191, 813-814, 822-823, 556, 828, 831-832, 836, 840, 844, 864, 871, 876, 878, 883, 884, 889-891) that are useful in the practice of the present invention to measure the expression pattern of the toxicity-related populations of genes (SEQ ID NOs: 1058-1238, 222, 224, 106, 226, 235, 237, 239, 246, 253, 258, 261, 270, 273, 274, 278, 111, 286, 302-304, 307, 308, 316-318, 322, 327, 119, 342, 358, 361, 367-368, 373, 381, 388, 401, 406, 409-410, 416-418, 423, 427-428, 430-432, 434, 439, 441, 447, 450, 455, 461, 464-465, 136, 137, 139, 474, 475, 482, 485, 488, 491, 492, 496, 500, 504, 524, 530, 534, 536, 541, 542, and 547).

Table 10 sets forth a mouse cell line toxicity-related population of genes (SEQ ID NOs: 1429-1448, 897, 901, 902, 919, 921, 922, 926, 928, 929, 931, 935, 939, 942, 943, and 946), and a population of oligonucleotide probes (SEQ ID NOs: 1449-1471, 952, 956, 957, 973, 975-976, 981, 983, 984, 986, 990, 999-1001, 1004-1007, and 1012-1014)

that are useful in the practice of the present invention to measure the expression pattern of the toxicity-related populations of genes (SEQ ID NOs: 1429-1448, 897, 901, 902, 919, 921, 922, 926, 928, 929, 931, 935, 939, 942, 943, and 946).

Table 12 sets forth a mouse cell line classifier population of genes (SEQ ID NOs: 1472-1730, 2, 896, 1429, 902, 1431, 1434, 15, 18, 19, 22, 25, 1436, 913, 1437, 916, 917, 920, 1441, 32, 923, 927, 39, 934, 935, 210, 939, 44, 1445, 943, 212, 946, 949), and a population of oligonucleotide probes (SEQ ID NOs: 1731-1996, 52, 951, 1450, 957, 1452, 1455, 65, 68, 69, 72, 75, 1457, 967, 1458, 970, 971, 974, 1462, 82, 977-978, 982, 90, 989, 990, 215, 1001, 999, 1000, 96, 1468, 1005-1006, 1970, 218, 1014, 1018, and 1019) that are useful in the practice of the present invention to measure the expression pattern of the classifier populations of genes (SEQ ID NOs: 1472-1730, 2, 896, 1429, 902, 1431, 1434, 15, 18, 19, 22, 25, 1436, 913, 1437, 916, 917, 920, 1441, 32, 923, 927, 39, 934, 935, 210, 939, 44, 1445, 943, 212, 946, 949).

Table 14 sets forth a mouse cell line population of genes (SEQ ID NOs: 1997-2795, 1473, 1475, 3, 1481, 1429, 1488, 1489, 1021, 1500, 902, 1515, 10, 1521, 13, 1538, 908, 1549, 1025, 1550, 1558, 1559, 1561, 1565, 21, 22, 1574, 912, 1614, 916-919, 1620, 1030, 1031, 922, 1639, 1645, 30, 1651, 35, 1673, 1674, 1682, 1033, 934, 1694, 936, 1034, 937, 210, 42, 939, 1444, 1698, 940, 209, 1703, 943, 1035, 945, 1710, 946, 1711, 1712, 1714, 948, 949, 142, 1728, and 49) that yield an expression pattern that correlates with the stimulation of PPAR α receptors by an agent, and a population of oligonucleotide probes (SEQ ID NO. 2796-3683, 1732, 1734, 53, 1740, 1449, 1450, 1747, 1748, 1037, 1759, 957, 1774, 60, 1780, 63, 1797, 962, 1808, 1041, 1809, 1817, 1818, 1820, 1824, 71, 72, 1833, 966, 1873, 970-973, 1879, 1046, 1047, 976, 1898, 1904, 80, 1910, 86, 1932, 1933, 1941, 1049, 989, 1953, 991-993, 1050, 1051, 994, 215, 216, 93, 94, 998-1001, 1465-1467, 1957, 1002, 214, 1962, 1005-1007, 1056, 1057, 1009-1014, 1974, 1975, 1977, 1979, 1016-1019, 1994, 101) that are useful in the practice of the present invention to measure the expression pattern of the foregoing populations of genes (SEQ ID NOs: 1997-2795, 1473, 1475, 3, 1481, 1429, 1488, 1489, 1021, 1500, 902, 1515, 10, 1521, 13, 1538, 908, 1549, 1025, 1550, 1558, 1559, 1561, 1565, 21, 22, 1574, 912, 1614, 916-919, 1620, 1030, 1031, 922, 1639, 1645, 30, 1651, 35, 1673, 1674, 1682, 1033, 934, 1694, 936, 1034, 937, 210, 42, 939, 1444, 1698, 940, 209, 1703, 943, 1035, 945, 1710, 946, 1711, 1712, 1714, 948, 949, 142, 1728, and 49).

Methods for identifying an efficacy-related population of genes or proteins: In

another aspect, the present invention provides methods for identifying an efficacy-related population of genes or proteins which are useful, for example, in the practice of the methods of the present invention for determining whether an agent possesses a defined biological activity. The methods of this aspect of the invention include the steps of (a) contacting a living thing with an agent that is known to elicit a desired biological response; and (b) identifying an efficacy-related population of genes or proteins in the living thing that yields an expression pattern that correlates with the occurrence of the desired biological response caused by the agent.

In some embodiments, the expression pattern of the efficacy-related population of genes or proteins appears in the living thing before the occurrence of the desired biological response caused by the agent. In some embodiments, the desired biological response does not occur in the living thing. For example, the living thing may be rat epididymal white adipose tissue which includes an efficacy-related population of genes, or proteins, that yields an expression pattern that correlates with the occurrence of a reduction in the concentration of glucose in rat's blood in response to a chemical agent administered to the rat. The expression pattern of the efficacy-related population of genes or proteins appears, however, before the reduction in blood glucose concentration.

Some embodiments of the methods of this aspect of the invention include the following steps: (a) measuring the level of expression of each member of a multiplicity of genes or proteins in the living thing, contacted with the agent, to yield a multiplicity of expression values; (b) measuring the level of expression of each member of the same multiplicity of genes or proteins in a reference living thing, that is not contacted with the agent, to yield a multiplicity of reference expression values; and (c) comparing the multiplicity of expression values with the multiplicity of reference expression values to identify an efficacy-related population of genes or proteins, wherein each individual gene or protein has an expression value in response to the agent that is significantly different from the corresponding reference expression value.

The reference living thing can be the living thing that is contacted with the agent before it is contacted with the agent. For example, a sample of cells or tissue may be removed from the living thing before it is contacted with the agent; thereafter, the living thing is contacted with the agent and a further sample of cells or tissue is removed from

the living thing, and gene expression is analyzed and compared between the two samples. The reference living thing can also be the same type of cells, tissue, organ or organism as the living thing contacted with the agent, except that the reference living thing is not contacted with the agent. For example, the living thing can be a db/db mouse to which is administered a dosage of rosiglitazone, and the reference living thing can be a different db/db mouse which is not administered a dosage of rosiglitazone. It is understood that typically a population of living things, and reference living things, are used in the practice of this aspect of the invention to provide a sufficiently large number of data for statistical analysis.

Some agents elicit more than one biological response in a living thing (*e.g.*, more than one desirable biological response, or more than one undesirable biological response, or at least one desirable biological response and at least one undesirable biological response). Elicitation of a biological response may require the action of a target molecule (*e.g.*, protein receptor). Typically, the target molecule is a component of a biochemical signal transduction pathway that is affected by the agent, and that conveys one, or more, biochemical signals (typically in the form of organic molecules, such as lipids) that elicit the biological response. For example, an agent may directly, physically, interact with a target molecule (*e.g.*, a protein receptor molecule located in a cell membrane) to elicit a desired biological response. Again by way of example, an agent may directly, physically, interact with a molecule, and this interaction may trigger the release of one or more signalling molecules that move within and/or between cells. One of these signalling molecules interacts with a target molecule (*e.g.*, a protein receptor molecule) to elicit a desired biological response.

A first target molecule may be required to elicit a first biological response when a living thing is contacted with an agent, and a second target molecule, that is different from the first target molecule, may be required to elicit a second biological response when the same living thing is contacted with the same agent. In one aspect, the present invention provides methods that can be used to identify an efficacy-related population of genes or proteins that yields an expression pattern that correlates with the occurrence of only the first or the second desired biological response caused by the direct, or indirect, interaction of the agent with one of two types of target molecules. These methods include the steps of (a) contacting the living thing with an agent that is known to elicit at least

two different desired biological responses in the living thing, wherein elicitation of a first desired biological response by the agent is mediated by a first target molecule, and elicitation of a second desired biological response by the agent is mediated by a second target molecule that is different from the first target molecule; (b) identifying an efficacy-related population of genes or proteins that yields an expression pattern that correlates with the occurrence of the first and second desired biological responses in response to the agent; (c) contacting a modified living thing with the agent, wherein the modified living thing is a member of the same species as the living thing and does not include any functional first target molecules; (d) identifying an efficacy-related population of genes or proteins that yields an expression pattern that correlates with the occurrence of the second desired biological response in the modified living thing in response to the agent; and (e) comparing the efficacy-related population of genes or proteins identified in step (b) with the efficacy-related population of genes or proteins identified in step (d) to identify an efficacy-related population of genes or proteins that yields an expression pattern that correlates with the occurrence of the first desired biological response caused by the agent.

It is understood that steps (a) through (d) can be in any temporal sequence (*e.g.*, steps (c) and (d) can be practised, to identify an efficacy-related population of genes or proteins that yields an expression pattern that correlates with the occurrence of the second target biological response, before steps (a) and (b) are practised to identify a population of genes or proteins that yields an expression pattern that correlates with the occurrence of the first and second target biological responses in response to the agent. The modified living thing can be, for example, a so-called "knockout" organism (or cells or tissues derived from a "knockout" organism) which has been genetically modified, for example by the process of targeted homologous recombination, to inactivate all genes encoding a target molecule.

Methods for identifying a toxicity-related population of genes or proteins: In another aspect, the present invention provides methods for identifying a toxicity-related population of genes or proteins which are useful, for example, in the practice of the methods of the present invention for determining whether an agent possesses a defined biological activity. The methods of this aspect of the invention include the steps of (a) contacting a living thing with an agent that is known to elicit an undesirable biological response; and (b) identifying a toxicity-related population of genes or proteins that yields

an expression pattern that correlates with the occurrence of the undesirable biological response caused by the agent.

In some embodiments, the expression pattern of the toxicity-related population of genes or proteins appears in the living thing before the occurrence of the undesirable biological response caused by the agent. In some embodiments, the undesirable biological response does not occur in the living thing.

Some embodiments of the methods of this aspect of the invention include the following steps: (a) measuring the level of expression of each member of a multiplicity of genes or proteins in the living thing, contacted with the agent, to yield a multiplicity of expression values; (b) measuring the level of expression of each member of the same multiplicity of genes or proteins in a reference living thing, that is not contacted with the agent, to yield a multiplicity of reference expression values; and (c) comparing the multiplicity of expression values with the multiplicity of reference expression values to identify a toxicity-related population of genes or proteins, wherein each individual gene or protein has an expression value in response to the agent that is significantly different from the corresponding reference expression value.

As described, *supra*, in connection with the methods of the invention for identifying an efficacy-related population of genes or proteins, the reference living thing can be the living thing that is contacted with the agent before it is contacted with the agent. The reference living thing can also be the same type of cells, tissue, organ or organism as the living thing contacted with the agent, except that the reference living thing is not contacted with the agent. It is understood that typically a population of living things, and reference living things, are used in the practice of this aspect of the invention to provide a sufficiently large number of data for statistical analysis.

Some embodiments of the methods of this aspect of the invention permit a user to distinguish between the expression pattern of an efficacy-related population of genes or proteins, and the expression pattern of a toxicity-related population of genes or proteins, wherein both expression patterns are caused by the same agent, and elicitation of the two expression patterns is mediated by two different target molecules. These embodiments include the steps of (a) contacting a living thing with an agent that is known to elicit a desirable biological response and an undesirable biological response in the living thing, wherein elicitation of the desirable biological response is mediated by a first target

molecule, and elicitation of the undesirable biological response is mediated by a second target molecule that is different from the first target molecule; (b) identifying a population of genes or proteins that yields an expression pattern that correlates with the occurrence of the desirable and undesirable biological responses caused by the agent; (c) 5 contacting a modified living thing with the agent, wherein the modified living thing is a member of the same species as the living thing and does not include any functional second target molecules; (d) identifying an efficacy-related population of genes or proteins that yields an expression pattern that correlates with the occurrence of the desirable biological response caused by the agent; and (e) comparing the population of 10 genes or proteins identified in step (b) with the efficacy-related population of genes or proteins identified in step (d) to identify a toxicity-related population of genes or proteins that yields an expression pattern that correlates with the occurrence of the undesirable biological response caused by the agent. By way of specific example, the first target molecule can be a PPAR γ receptor and the second target molecule can be a PPAR α 15 receptor.

In the context of the methods of this aspect of the invention, the terms "elicitation of the desirable biological response is mediated by a first target molecule" and "elicitation of the undesirable biological response is mediated by a second target molecule" mean that the target molecule is a component of the biochemical signal transduction pathway that is 20 affected by the agent, and that conveys one, or more, biochemical signals (typically in the form of organic molecules, such as lipids) that elicit the desirable, or undesirable, biological response.

It is understood that steps (a) through (d) can be in any temporal sequence. The modified living thing can be, for example, a so-called "knockout" organism (or cells or 25 tissues derived from a "knockout" organism) which has been genetically modified, by the process of targeted homologous recombination, to inactivate all genes encoding a target molecule.

Methods for identifying a classifier population of genes or proteins: In another aspect, the present invention provides methods for identifying a classifier population of 30 genes or proteins, which are useful, for example, in the practice of the methods of the present invention for determining whether an agent possesses a defined biological activity. The methods of this aspect of the invention include the steps of (a) contacting a

living thing with a first reference agent that is known to cause a first biological response; (b) identifying a first population of genes or proteins that yields an expression pattern that correlates with the occurrence of the first biological response caused by the first reference agent; (c) contacting a living thing with a second reference agent that is known to cause a second biological response, wherein the living thing is the same living thing that is contacted with the first reference agent, or is a different living thing that is a member of the same species as the living thing that is contacted with the first reference agent; (d) identifying a second population of genes or proteins that yields an expression pattern that correlates with the occurrence of the second biological response caused by the second reference agent; and (e) comparing the first population of genes or proteins to the second population of genes or proteins and thereby identifying a classifier population of genes or proteins that produces an expression pattern that most clearly distinguishes between the first reference agent and the second reference agent. It is understood that the combination of step (a) and step (b) can be performed before, during or after the combination of step (c) and step (d).

The following examples merely illustrate the best mode now contemplated for practicing the invention, but should not be construed to limit the invention.

EXAMPLE 1

This Example describes the identification of two efficacy-related populations of genes that are both useful in the practice of the methods of the invention for identifying agonists and partial agonists of PPAR γ . One efficacy-related population of 50 genes was identified in mouse EWAT tissue. The nucleotide sequences of these 50 genes are set forth in the portion of this patent application entitled SEQUENCE LISTING and are identified in Table 1, (SEQ ID NOs: 1-50). The nucleotide sequences of the 52 oligonucleotide probes used to measure the expression levels of these 50 genes (SEQ ID NOs: 1-50) are set forth in the SEQUENCE LISTING and identified in Table 1, (SEQ ID NOs: 51-102). The other efficacy-related population of genes includes 21 genes that were identified in cultured 3T3L1 mouse adipocyte cells (passages 3-9). These 21 genes, whose nucleotide sequences are set forth in the SEQUENCE LISTING (SEQ ID NOs: 2, 3, 8, 9, 15, 16, 18, 19, 21, 23, 25, 26, 28, 32, 35, 37-39, 42, 44, 49), are a subset of the foregoing 50 genes. The oligonucleotide probes used to measure the expression levels of these 21 genes (SEQ ID NOs: 2, 3, 8, 9, 15, 16, 18, 19, 21, 23, 25, 26, 28, 32, 35, 37-39,

42, 44, 49) are identified in Table 2, (SEQ ID NOs: 52, 53, 58, 59, 65, 66, 68, 69, 71, 73, 75, 76, 78, 82, 86, 88-90, 93, 94, 96, 101).

5 Table 1. PPAR γ _Mouse_Efficacy_Probe_52 (Species: db/db Mouse)

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
AK010455	2410008K03Rik	1	51
AW909114	MGC28611	2	52
NM_008543	Madh7	3	53
AF282730	Timp4	4	54
M12347	Acta1	5	55
NM_007377	Aatk	6	56
AK002237	Gadd45g	7	57
NM_030701	Pumag-pending	8	58
AK012169	Slitl2	9	59
AV279434	4930458D05Rik	10	60
NM_022020	Rbp7	11	61
NM_019738	Nupr1	12	62
AK004867	1300002P22Rik	13	63
AK015355	4930442A21Rik	14	64
AK009315	2310012G06Rik	15	65
AJ277212	hypothetical protein	16	66
NM_026167	1200009K10Rik	17	67
NM_011782	Adamts5	18	68
NM_020578	Ehd3	19	69
NM_016873	Wisp2	20	70
AV280352	AV280352	21	71
AK010891	2510002J07Rik	22	72
AK020638	9530072E15Rik	23	73
AK018128	6330406I15Rik	24	74
AK004732	1200013A08Rik	25	75

BC004720	MGC36388	26	76
NM_026252	4930447D24Rik	27	77
NM_031180	Klb-pending	28	78
NM_020025	B3galt2	29	79
AK004897	FacI2	30	80
AK016444	4931408D14Rik	31	81
AK013740	6530401D17Rik	32	82
AF090738	Irs2	33	83
			84
AK004293	2310041C05Rik	34	85
BC003479	LOC216820	35	86
AK018673	Mrpl19	36	87
AB001735	Adamts1	37	88
AK018423	8430417G17Rik	38	89
AK016103	4930553F04Rik	39	90
BC003755	Eya2	40	91
BB265432	BB265432	41	92
NM_013743	Pdk4	42	93
			94
U03560	Hsp25	43	95
J04632	Gstm1	44	96
L12447	Igfbp5	45	97
M21855	Cyp2b9	46	98
AI467229	Ppp1r3a	47	99
X13297	Acta2	48	100
Z37107	Ephx2	49	101
AW146087	BB104597	50	102

Table 2. PPAR γ _3T3L1_Efficacy_Probe_22 (Species: Mouse Cell Line)
(A subset of Table_1: PPAR γ _Mouse_Efficacy_Probe_52 (Species: db/db Mouse))

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
AW909114	MGC28611	2	52
NM_008543	Madh7	3	53
NM_030701	Pumag-pending	8	58
AK012169	Slit2	9	59
AK009315	2310012G06Rik	15	65
AJ277212	hypothetical protein	16	66
NM_011782	Adamts5	18	68
NM_020578	Ehd3	19	69
AV280352	AV280352	21	71
AK020638	9530072E15Rik	23	73
AK004732	1200013A08Rik	25	75
BC004720	MGC36388	26	76
NM_031180	Klb-pending	28	78
AK013740	6530401D17Rik	32	82
BC003479	LOC216820	35	86
AB001735	Adamts1	37	88
AK018423	8430417G17Rik	38	89
AK016103	4930553F04Rik	39	90
NM_013743	Pdk4	42	93 94
J04632	Gstm1	44	96
Z37107	Ephx2	49	101

Genetically altered, diabetic, mice (db/db strain, available from the Jackson Laboratory, Bar Harbor, ME, U.S.A., as strain C57B1/KFJ, and described by Chen et al., *Cell* 84: 491-495 (1996), and by Combs et al., *Endocrinology* 142: 998-1007 (2002)), and lean mice, were administered one of two PPAR γ agonists, either Rosiglitazone (5-(4-{2-[methyl(pyridin-2-yl)amino]ethoxy}benzyl)-1,3-thiazolidine-2,4-dione) or {2-[2-(4-phenoxy-2-propylphenoxy)ethyl]-1*H*-indol-5-yl}acetic acid. The PPAR γ agonists were

orally administered once per day for a period of two days or eight days at a dosage of 10 milligrams per kilogram body weight. EWAT tissue was removed from the treated mice six hours after administration of the second or eighth dose. Both of the treatments were divided into four groups:

5 Group 1: db/db vehicle control vs. db/db vehicle control pool (the control pool included all of the mice that were administered the vehicle alone without any PPAR γ agonist).

 Group 2: lean mouse vs. db/db vehicle control pool.

 Group 3: db/db vehicle control pool vs. Rosiglitazone-treated db/db mice.

10 Group 4: db/db vehicle control pool vs. db/db mice treated with {2-[2-(4-phenoxy-2-propylphenoxy)ethyl]-1*H*-indol-5-yl}acetic acid.

 A hybrid ANOVA method was used to compute the pvalue (hereafter ANOVA-pvalue) for the null hypothesis that the genes are not differentially regulated within each group. Standard ANOVA estimates the variance within a group by the spread of replicates within each group. The error of the variance within a group can be large when
15 the number of replicates in each group is small, thereby yielding more false positives (mistakenly identifying a non-significant difference between groups as being significant). This problem is avoided by using the hybrid ANOVA method to estimate the error within a group. The variance within a group comes from at least two sources: sample variance
20 and measurement error (platform variance). The Hybrid-ANOVA sets a low limit of the within-group variance to the platform variance. The platform variance is estimated from previous replicates with similar gene expression levels.

 Signature genes were identified for each of the four groups (*i.e.*, genes that showed significant, differential, expression in the comparison made in each of the four
25 groups). Based upon the two day data (each treatment was repeated five times), each probe having an ANOVA-pvalue smaller than 0.01, and having an absolute value of the mean of the logRatio greater than log₁₀ 1.5 was considered to be a signature gene for each group.

 First, the signature genes in Groups 3 and 4 were united. Then the united
30 signature genes from Groups 3 and 4 were compared with the signature genes from Group 2, and the overlapping population of genes between the two compared groups was identified. Then the genes within the overlapping population that were regulated in the

opposite direction in the united signature gene population compared to the Group 2 signature gene population were identified (*e.g.*, genes that are differentially expressed at a higher, or lower, level in the db/db mice, but are differentially expressed at a lower, or higher, level in mice treated with a PPAR γ agonist are likely to be markers for the desired effect of reducing blood glucose level).

Finally, artifactual signature genes in Group 1 were removed from the resulting set. The artifactual signature genes are those genes that were differentially regulated in Group 1, and so represented the variation in gene expression between animals. A total of 52 probes (SEQ ID NOs: 51-102) were thereby identified as the efficacy reporter population in the EWAT tissue of db/db mice treated with the PPAR γ agonists. These 52 probes (SEQ ID NOs: 51-102) corresponded to 50 genes (SEQ ID NOs: 1-50). These 50 genes (SEQ ID NOs: 1-50) are useful in the practice of the present invention as an efficacy-related population of genes to identify PPAR γ agonists and/or PPAR γ partial agonists using mouse EWAT tissue.

The usefulness of the 50 genes (SEQ ID NOs: 1-50), as an efficacy-related population of genes to identify PPAR γ agonists and/or PPAR γ partial agonists, was confirmed by using the data from the treatments lasting for seven days in which eight doses were administered to the animals (the first dose being administered at day zero) to determine whether the expression of the 50 genes (SEQ ID NOs: 1-50), corresponding to the 52 probes (SEQ ID NOs: 52-102), correlated with the desired biological end point (*i.e.*, lowering of glucose concentration in blood plasma).

The reduction in the concentration of glucose in blood plasma was measured for each mouse in the study. The correlation coefficient of the logRatio of each of the 52 probes (SEQ ID NOs: 52-102) with the end point data was calculated. Probes with correlation coefficient of more than 0.5 were selected. All 52 probes (SEQ ID NOs: 52-102) were found to have a satisfactory correlation coefficient (more than 0.5) with the end point data.

The 52 probes (SEQ ID NOs: 52-102) were also mapped onto the gene expression profiles of mouse 3T3L1 adipocyte cells, cultured *in vitro*, that had been treated with either Rosiglitazone (at an effective concentration of 600 nM) or {2-[2-(4-phenoxy-2-propylphenoxy)ethyl]-1*H*-indol-5-yl}acetic acid (at an effective concentration of 3870 nM). Twenty four hours after the cells were contacted with one or other of the

foregoing agents the cells were harvested and RNA extracted therefrom. Twenty two probes (SEQ ID NOs: 52, 53, 58, 59, 65, 66, 68, 69, 71, 73, 75, 76, 78, 82, 86, 88-90, 93, 94, 96, 101) were identified that were differentially regulated in the 3T3L1 adipocytes in response to both of the foregoing agents. These 22 probes (SEQ ID NOs: 52, 53, 58, 59, 65, 66, 68, 69, 71, 73, 75, 76, 78, 82, 86, 88-90, 93, 94, 96, 101) corresponded to 21 genes (two probes hybridized to the same gene) (SEQ ID NOs: 2, 3, 8, 9, 15, 16, 18, 19, 21, 23, 25, 26, 28, 32, 35, 37-39, 42, 44, 49). These 21 genes (SEQ ID NOs: 2, 3, 8, 9, 15, 16, 18, 19, 21, 23, 25, 26, 28, 32, 35, 37-39, 42, 44, 49) are useful in the practice of the present invention as an efficacy-related population of genes to identify PPAR γ agonists and/or PPAR γ partial agonists using the 3T3L1 mouse cell line.

The expression data for the 21 genes (SEQ ID NOs: 2, 3, 8, 9, 15, 16, 18, 19, 21, 23, 25, 26, 28, 32, 35, 37-39, 42, 44, 49) in response to Rosiglitazone and PPAR γ agonist {2-[2-(4-phenoxy-2-propylphenoxy)ethyl]-1*H*-indol-5-yl}acetic acid were averaged and treated as a vector for the full template. Thus, an efficacy value a PPAR γ agonist, or partial agonist, was calculated in the following manner. The value (expressed as a percentage) of the logRatio divided by the template logRatio for each of the 22 probes (SEQ ID NOs: 52, 53, 58, 59, 65, 66, 68, 69, 71, 73, 75, 76, 78, 82, 86, 88-90, 93, 94, 96, 101) was calculated, and then the mean of the resulting 22 percentages was calculated. This mean value was the PPAR γ efficacy value for the PPAR γ agonist, or partial agonist.

A chi-square fitting was also used to calculate the efficacy value for each tested PPAR γ agonist, or partial agonist. The chi-square fitting formula used was:

$$\chi^2 = \sum_{i=1}^{22} (S * R_i - X_i)^2 / (\sigma_{Ri}^2 + \sigma_{Xi}^2)$$

Where R_i , σ_{Ri} stand for the logRatio and error for logRatio of the full template. X_i and σ_{Xi} stand for the logRatio and error for logRatio of the testing compound. This chi-square fitting method is described, for example, by W. Press et al., Numerical Recipes in C, Chapter 14, Cambridge University Press (1991).

A very similar result was obtained using each method for calculating the efficacy values (the correlation coefficient for the scores calculated by the two methods was 0.9996).

Table 3 shows the efficacy scores for full or partial agonists of PPAR γ . A PPAR α agonist was included as a control.

TABLE 3.

Compound	Efficacy Score
Agonist 1	1.033
Agonist Rosiglitazone	0.967
Partial agonist 15	0.795
Partial agonist 16	0.776
Partial agonist 17	0.644
Partial agonist 4	0.578
Partial agonist (2 <i>R</i>)-2-(4-chloro-3-{[3-(6-methoxy-1,2-benzisoxazol-3-yl)-2-methyl-6-(trifluoromethoxy)-1 <i>H</i> -indol-1-yl]methyl}phenoxy)propanoate	0.561
Partial agonist 10	0.511
Partial agonist 12	0.469
Partial agonist 9	0.463
Partial agonist 11	0.447
Partial agonist 14	0.376
Partial agonist 13	0.367
PPAR α agonist	0.178

EXAMPLE 2

This Example describes the identification of toxicity-related populations of genes that are useful in the practice of the methods of the invention for evaluating the toxic, or
5 otherwise undesirable, biological activities of agonists and partial agonists of PPAR γ .

Measuring the Toxic Effects of PPAR γ Agonists and PPAR γ Partial Agonists in Rats: Eleven PPAR γ agonists or partial agonists were tested in rats in an experiment that was divided into several experiments (referred to as phases) because the design of the overall experiment required the use of more rats than could be handled in a single
10 experiment. Each phase of the experiment tested 3 compounds, with rosiglitazone present in every phase as a bridging compound. For each compound, 3 doses were selected that represented the effective dose (EC₅₀) in db/db mice, as well as 1/3 and 3 times the EC₅₀. Eight animals were treated per dose and per compound. The treatments

lasted 7 days, and a PPAR γ agonist or partial agonist was administered once per day. Animals were sacrificed 24 hours, or later, after the last dose of the treatment, so that the plasma volume data could be measured. Heart, kidney and EWAT tissues from phases 5, 7, 8 and 9 were collected. For phase 4, only heart tissues were available. Heart weight, body weight and plasma volume data were recorded for each animal.

Microarray profiling: Heart, kidney and EWAT tissues were profiled using gene microarrays to identify genes that are toxicity biomarkers. Tissues from the animals treated only with the vehicle (that did not include a PPAR γ agonist or partial agonist) were used as the reference channel for the microarray profiling. cDNA made from RNA extracted from tissues from animals treated with a PPAR γ agonist, or partial agonist, were labeled with different fluorophores and competitively hybridized with the reference sample on the same array. Approximately 25,000 rat genes had representative oligonucleotide probes on the array. To save the array budget, only a subset of animals were profiled for some phases. When selecting the subset of animals for profiling, efforts were made to avoid biases by choosing animals covering a broad range of biological endpoints. In those phases where a subset were selected, 3 out of 8 rats were selected from the low and medium dose, 6 out of 8 rats were selected from the high dose. It was assumed that effects associated with the high dose were more likely to be drug effects.

Methods for Identifying Toxicity-Related Genes: Genes were selected whose expression correlated with heart weight increase and/or plasma volume expansion. A dimension reduction approach was also taken to address the statistical overfitting problem. Since there were 25,000 probes printed on the microarray, it was possible to mistakenly select a few genes, by chance, whose expression appeared to be correlated with the biological end point of interest. This is referred to as the overfitting problem. The following approach was used to address the overfitting problem. Regulated genes were identified by first identifying robust signature genes for each compound (*i.e.*, genes whose expression was consistently affected by the compound being tested). The union of the signature genes for all of the compounds tested was clustered into subgroups, and the groups of genes whose expression pattern correlated with the biological endpoint were identified. Since the number of subgroups was usually small (around 4 subgroups), there was no danger of overfitting. This Example describes application of these methods to

identifying genes that are markers for increased heart weight in response to a PPAR γ agonist or partial agonist.

(1) Correlating an Increase in Heart Weight with the Expression of Individual Genes in Rat Hearts: Data sets used to identify the correlation were from phases 5, 7, and 8. Gene expression was correlated with an increase in heart weight observed in rats by selecting genes significantly regulated ($P < 0.01$) in more than 3 experiments in each data set. These genes were called the signature genes. The correlation between the log(ratio) of each of the signature genes and the increase in heart weight were calculated for each data set. In this experiment the heart weight was normalized to the body weight. Since the data set for phases 7 and 8 were relatively small, phase 7 data and phase 8 data were also combined for the above calculations, in addition to being used separately. Signature genes were selected that had a magnitude of correlation greater than 0.3 from each data set.

There were almost no overlapping genes from more than four data sets when the individual animal heart weight data was used. To reduce possible heart weight data measurement error, and to emphasize the drug related toxicity effect, the heart weight data from eight animals (irrespective of whether the animals had been profiled using the microarray) of each treatment group were averaged and used as the toxicity measurement. Using the average endpoint data, 10 overlapping genes were identified.

Since the magnitude of correlation threshold of 0.3 was arbitrary, and the number of overlapping genes was relatively small, the overlapping genes were used as the seed genes to identify similarly regulated genes in data from phases 5 and the combination of phases 7 plus 8. Genes whose regulation correlated with any of the 10 overlapping genes in either the data from phase 5 or the data from the combination of phases 7 plus 8, with a magnitude of correlation greater than 0.8, were selected. Sixty three probes were thereby identified as toxicity-related genes that indicate an undesirable increase in heart weight.

It was possible just by chance to incorrectly select a few toxicity-related genes since there were 25,000 genes present on the microarray. Therefore it was important to have some test data sets (which were not involved in the toxicity-related gene selection) to validate the toxicity-related genes.

(2) Using Strongly Regulated Genes to Identify a Toxicity Related Gene Population: Selecting toxicity-related genes based on the analysis of individual signature

gene expression patterns was the most sensitive method to identify a toxicity-related gene population, but also had the highest risk of over-fitting, because of the high degree of freedom. The statistical significance was discounted by the big Bonferroni correction factor. The separate experiments were not fully independent from each other, since a bridging compound was used (rosiglitazone). Therefore a dimension reduction was used to reduce the risk of over-fitting.

First, robust signature genes (*i.e.*, genes whose expression was consistently affected by the compound being tested and which correlated with the target biological effect) were identified in response to each PPAR γ agonist, or partial agonist ($P < 0.01$ and amplitude of $\log(\text{ratio}) > 0.15$ in at least 80% of the replicates of any treatment, same direction of regulation across multiple doses within a drug, but not in any of the control experiments with $\log(\text{ratio}) > 0.2$). Then the union of drug signature genes from each phase was analyzed to identify the signature genes that appear in more than one phase. The signature genes from all phases were clustered into a finite number of patterns (< 10), and the patterns associated with increased heart weight were identified. The heart tissues from phases 5, 7, 8, 9 were used for selecting the robust signature genes.

A total of 114 signature genes were selected from all phases. Gene dimension clustering showed that two groups of genes (one up-regulated and one down-regulated) correlated with increased heart weight. The degree of the correlation of these two groups of genes with increased heart weight was further verified by calculating the correlation coefficient between the mean $\log(\text{ratio})$ of the up-regulated (or down-regulated) group with the heart weight. The correlations were 0.75 or higher. The chance probability of having such high correlation by random fluctuation was at the level of 2×10^{-7} .

Combining the Results of the Gene Expression Analysis Described in Sections (1) and (2): A set of 48 probes were selected from the 114 probes identified in Section (2). Combining these 48 probes with the 63 probes identified as described in Section (1) yielded a total of 85 unique probes. These probes were screened again to identify those probes having a correlation coefficient between gene expression and increase in heart weight greater than 0.4. This process resulted in the final 55 probes. The nucleotide sequence identification numbers of these 55 probes are identified in Table 4, (SEQ ID NOs: 153-207). These 55 probes (SEQ ID NOs: 153-207) corresponded to 50 different genes. The nucleotide sequence identification numbers of these 50 genes are identified in

Table 4, (SEQ ID NOs: 103-152). These 50 genes (SEQ ID NOs: 103-152) are useful in the practice of the present invention as a toxicity-related gene population.

Table 4. PPAR γ _Rat_Heart_Toxicity_HeartWeight_Probe_55 (Species: Rat)

5

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
AB011365	Pparg	103	153 154
D16478	Hadha	104	155
J02791	Acadm	105	156 157
Y09333	Mtel	106	158
AI230591	g3814478	107	159
AI105094	g3709266	108	160
AA891470	g3708538	109	161
AI059241	g3333018	110	162
G3638603	g3638603	111	163
AA859032	g2948383	112	164
BF288765	g3726475	113	165
AI071468	g3397683	114	166
G3817698	g3817698	115	167
AI070283	Pcsk4	116	168
G3189597	g3189597	117	169
g3815735	g3815735	118	170
AI170067	g3710107	119	171
AI407765	g3707790	120	172
AI170387	g3710427	121	173
AI231193	g3815073	122	174
g979428	g979428	123	175
G3105928	g3105928	124	176
AI411979	g3072442	125	177

600523591R1	600523591R1	126	178
AA964752	g3138244	127	179
AI009219	g3223051	128	180
BE101435	g2937230	129	181
AI044576	g3291437	130	182
G3036695	g3036695	131	183
BG372920	g3189161	132	184
AI105417	g3709501	133	185
AI177360	g3727998	134	186
G3189544	g3189544	135	187
AI227820	Mgll	136	188
AA892864	Mgll	137	189
BF395162	g3223602	138	190
G977669	g977669	139	191
g4135065	g4135065	140	192
M23601	Maob	141	193
L23108*	Cd36	142	194
U75581	Fabp4	143	195 196 197
NM_012778	Aqp1	144	198
U41453	Akap12	145	199
U67863	Mc4r	146	200 201
NM_031315	Ctel	147	202
NM_013120	Gckr	148	203
NM_017306	Dci	149	204
NM_022594	Ech1	150	205
D00729	D00729	151	206
NM_021751	Prom	152	207

*Mouse gene sequence L23108 (SEQ ID NO:142) and corresponding mouse probe (SEQ ID NO:194) were used to measure gene expression of the rat homolog(s) to mouse Cd36 gene.

5 Identifying a Toxicity-Related Gene Population in Mice that are Early Predictors for Increased Heart Weight: The 55 probes (SEQ ID NOs: 153-207) corresponding to the toxicity-related population of 50 genes (SEQ ID NOs: 103-152), described in the preceding paragraph, were further analyzed to identify a sub-population of genes that are useful as early biomarkers for the onset of the adverse effect of heart weight increase due
10 to administration of a PPAR γ agonist or partial agonist.

In order to find the early biomarkers, the 55 probes (SEQ ID NOs: 153-207) were mapped onto an earlier data set, obtained by treating mice with PPAR γ agonists and partial agonists. This earlier experiment was referred to as the "747 tissue experiment" since 747 tissues were collected. PPAR γ agonists Rosiglitazone and 5-[4-(3-{4-[4-
15 (methylsulfonyl)phenoxy]-2-propylphenoxy}propoxy)phenyl]-1,3-thiazolidine-2,4-dione were administered to mice once per day for one to seven days. Tissues were removed 6 hours after the most recent dose of PPAR γ agonist from animals with 1, 2, 4 and 8 treatments (note that the first dosage was administered at time zero and tissues were removed from the treated animals six hours later; thus, the animals sacrificed at 7 days
20 had received 8 treatments). By mapping the 55 rat probes (SEQ ID NOs: 153-207) into this set of mice data, and also requiring genes to be regulated by just one or two treatments, five early biomarkers were identified that were useful early reporters of heart toxicity. The nucleotide sequences of these 6 probes (SEQ ID NOs: 213-218), corresponding to 5 genes (SEQ ID NOs: 208-212), as identified in Table 5.

Table 5. PPAR γ _Mouse_Heart_EarlyBiomarkers_ForHeartWeight_Probe_5 (species Mouse)

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
AK003305	1110002J19Rik	208	213
AJ001118	Mgll	209	214
M13264	Fabp4	210	215 216
L02914	Aqp1	211	217
U01841	Pparg	212	218

5

These early biomarkers are also useful as a toxicity-related gene population in the practice of the present invention. The use of these early biomarkers helps to identify those candidate PPAR γ agonists and/or partial agonists that possess the undesirable property of causing an increase in heart weight.

10 Heart Weight Biomarkers in EWAT: EWAT is a target tissue for the PPAR γ agonists, and is a useful tissue for microarray profiling because it has a high signal to noise ratio. In addition, it is advantageous to be able to assess both efficacy and toxicity using the same tissue.

15 Approximately 1800 robust signature genes were selected (using data from phases 5, 7, 8 and 9). The log(ratio)s of the 1800 robust EWAT signature genes were directly correlated with heart weight. 355 Probes were identified, from the population of 1800 robust probes, that had a correlation value of at least 0.6. The correlation value was a measure of correlation between expression of the gene corresponding to the probe and an increase in heart weight. The identities of these 355 probes are given in Table 6 (SEQ ID
20 NOs: 551-894, 155, 157, 164, 171, 178, 179, 185, 188, 204-206). These 355 probes (SEQ ID NOs: 551-894, 155, 157, 164, 171, 178, 179, 185, 188, 204-206) corresponded to 343 different genes that are identified in Table 6 (SEQ ID NOs: 219-550, 104, 105, 112, 119, 126, 127, 133, 136, 149-151).

Table 6. PPAR γ _Rat_eWAT_Toxicity_HeartWeight_Probe_355 (Species: Rat)

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
AA956114		219	551
D00688	Maoa	220	552 553
D16478	Hadha	104	155
J02791	Acadm	105	157
J05029	Acadl	221	554 555 556
K03249	Ehhadh	222	557 558 559
M22756	Ndufv2	223	560
M29853	Cyp4b1	224	561 562 563
G3292626	g3292626	225	564
A1170251	g3710291	226	565
A1411835	g3019978	227	566
A1229166	g3813053	228	567
G3667853	g3667853	229	568
AA891248	g3018127	230	569
G3731024	g3731024	231	570
BF282327	g3812938	232	571
AA944463	g3104379	233	572
G3704882	g3704882	234	573
A1113016	g3512965	235	574

AW142276	g3815698	236	575
G3103828	g3103828	237	576
700034842H1	700034842H1	238	577
AI408705	g2863227	239	578
G3227498	g3227498	240	579
G3291499	g3291499	241	580
AI030918	g3248744	242	581
G3712254	g3712254	243	582
G3728605	g3728605	244	583
G979167	g979167	245	584
G3189034	g3189034	246	585
G3018667	g3018667	247	586
G3188003	g3188003	248	587
AI170000	g3710040	249	588
X57405	Notch1	250	589
G979644	g979644	251	590
G3712007	g3712007	252	591
AI144876	Ass	253	592
AI235475	g3828981	254	593
AW915407	g2938925	255	594
BF288349	g2938279	256	595
AI228128	g3812015	257	596
AI411031	g3709121	258	597
AI168968	g3705276	259	598
BF398271	g3292264	260	599
G2862965	g2862965	261	600
G807326	g807326	262	601
G4133385	g4133385	263	602
BE107150	g2939171	264	603
AI044760	g3291621	265	604
BF400209	g3226969	266	605

G3705573	g3705573	267	606
BF283751	g4132683	268	607
AI411520	g4134016	269	608
BF560807	g3187199	270	609
G3221992	g3221992	271	610
G4131482	g4131482	272	611
G3071873	g3071873	273	612
AA799476	g2862431	274	613
G977129	g977129	275	614
g3399275	g3399275	276	615
G3729761	g3729761	277	616
AI411212	g3710380	278	617
AI180004	g3730642	279	618
AI411375	g2939160	280	619
G3223977	g3223977	281	620
BE116768	g3638204	282	621
BF282695	g3511588	283	622
701347850H1	701347850H1	284	623
G3709587	g3709587	285	624
G3813131	g3813131	286	625
AI603127	g3222358	287	626
G3223106	g3223106	288	627
AA859032	g2948383	112	164
G3225430	g3225430	289	628
G3019722	g3019722	290	629
g3292396	g3292396	291	630
AI599484	g3119754	292	631
BE110616	g3726615	293	632
G3187488	g3187488	294	633
AI044912	g3291731	295	634
AI511066	g3667675	296	635

AA891689	g3018568	297	636
AA799829	g4131444	298	637
AI101639	g3706514	299	638
AI013110	g3227166	300	639
G3019363	g3019363	301	640
g3636884	g3636884	302	641
BF284475	g3711260	303	642
AA894090	g3020969	304	643
G2863149	g2863149	305	644
G977018	g977018	306	645
BE113034	g3815452	307	646
G3137782	g3137782	308	647
700064632H1	700064632H1	309	648
G3292491	g3292491	310	649
AI599819	g3120109	311	650
AI233766	g3817646	312	651
700508236H1	700508236H1	313	652
701347935H1	701347935H1	314	653
g2937470	g2937470	315	654
AI170808	g3710848	316	655
G3727129	g3727129	317	656
AW528443	g4136134	318	657
AI235135	g3828641	319	658
G3511674	g3511674	320	659
BG372437	g4135897	321	660
BF556962	g3708808	322	661
AI144760	g3666559	323	662
AI598414	g3396210	324	663
g3118749	g3118749	325	664
AI511051	g3511894	326	665
AA963069	g3136561	327	666

G3729474	g3729474	328	667
G3709332	g3709332	329	668
BF288286	g2937985	330	669
AI170067	g3710107	119	171
AI175045	g3725683	331	670
BG373072	g3816835	332	671
BF405032	g3035182	333	672
G4134345	g4134345	334	673
BG373122	g978418	335	674
BG381583	g4132471	336	675
G2863503	g2863503	337	676
BF281235	g3121225	338	677
AA892281	g3019160	339	678
AI168935	g4134349	340	679
G3223313	g3223313	341	680
AA998205	g3188856	342	681
G3705112	g3705112	343	682
AA799656	g2862611	344	683
701219674H1	701219674H1	345	684
G3103230	g3103230	346	685
AA998461	g3189112	347	686
BG378631	g3729576	348	687
AW525026	g3246829	349	688
AA964882	g3138374	350	689
G3513255	g3513255	351	690
AI009759	g3223591	352	691
BG378729	g3104259	353	692
BF283386	g3121114	354	693
AW915566	g2864131	355	694
BF288366	g2938368	356	695
g2864124	g2864124	357	696

701216507H1	701216507H1	358	697
G2937254	g2937254	359	698
AA892593	g3019472	360	699
BG377008	g2863410	361	700
AI231886	g3815766	362	701
AI406687	g3019436	363	702
AI137895	g3638672	364	703
BF558361	g3706834	365	704
AI060312	g3334089	366	705
AI058968	g3332745	367	706
701349156H1	701349156H1	368	707
700032770H1	700032770H1	369	708
701220604H1	701220604H1	370	709
701222864H1	701222864H1	371	710
701218584H1	701218584H1	372	711
700508607H1	700508607H1	373	712
G979526	g979526	374	713
600507145R1	600507145R1	375	714
600513733R1	600513733R1	376	715
600521564R1	600521564R1	377	716
G979217	g979217	378	717
600521930R1	600521930R1	379	718
600511860R1	600511860R1	380	719
600512417R1	600512417R1	381	720
701417945H1	701417945H1	382	721
600516384R1	600516384R1	383	722
G3711582	g3711582	384	723
600516355R1	600516355R1	385	724
600511327R1	600511327R1	386	725
AI600147	600521079R1	387	726
G4134738	g4134738	388	727

G3727115	g3727115	389	728
600521206R1	600521206R1	390	729
AA819547	g2889636	391	730
BF281400	g2672900	392	731
600523591R1	600523591R1	126	178
600521690R1	600521690R1	393	732
600510887R1	600510887R1	394	733
AI175980	600512928R1	395	734
AA944036	g3103952	396	735
600518269R1	600518269R1	397	736
AI175479	600513115R1	398	737
G3188371	g3188371	399	738
700692105H1	700692105H1	400	739
G3225638	g3225638	401	740
600507783R1	600507783R1	402	741
S74321	cytochrome bc- l complex core P	403	742
BE109568	600509475R1	404	743
G3071118	g3071118	405	744
AI010433	Cdtw1	406	745
G2938798	g2938798	407	746
AA866477	g2961938	408	747
BG381033	g4131620	409	748
600512426R1	600512426R1	410	749
600509794R1	600509794R1	411	750
G2862597	g2862597	412	751
XM341383	Pcca	413	752
AI228236	g3812123	414	753
600512874R1	600512874R1	415	754
G4134262	g4134262	416	755
600523104R1	600523104R1	417	756

600520906R1	600520906R1	418	757
G4131829	g4131829	419	758
AI231810	g3815690	420	759
AI072712	600507095R1	421	760
600515268R1	600515268R1	422	761
G3815486	g3815486	423	762
600509881R1	600509881R1	424	763
AI232494	g3816374	425	764
AA964752	g3138244	127	179 ✓
AI410548	g3073005	426	765
G3104296	g3104296	427	766
600514084R1	600514084R1	428	767
600519478R1	600519478R1	429	768
600508574R1	600508574R1	430	769
AA875107	g2980055	431	770
AI104528	g3708870	432	771
G3227353	g3227353	433	772
AI171656	g3711696	434	773
G2863419	g2863419	435	774
BE102621	g3512812	436	775
G3398286	g3398286	437	776
g3830855	g3830855	438	777
AI104348	g3708719	439	778
AI599410	g2889576	440	779
G3831232	g3831232	441	780
AI145507	g3667306	442	781
G3396295	g3396295	443	782
AA891814	g3018693	444	783
G4133678	g4133678	445	784
AW434257	g3397092	446	785
G3019879	g3019879	447	786

G3018575	g3018575	448	787
AI412460	g3704629	449	788
BG381624	g3018621	450	789
AW142969	g3727595	451	790
G978652	g978652	452	791
AI105417	g3709501	133	185
AI072493	g3398687	453	792
G2862397	g2862397	454	793
AA800782	g4131537	455	794
AI171367	g3711407	456	795
BE111132	g3397248	457	796
G977490	g977490	458	797
700585804H1	700585804H1	459	798
BF288776	g3726534	460	799
G4135910	g4135910	461	800
G979011	g979011	462	801
BG374035	g3726504	463	802
G978793	g978793	464	803
G3707669	g3707669	465	804
701350526H1	701350526H1	466	805
701216526H1	701216526H1	467	806
AI227820	MgII	136	188
BE103080	g3811971	468	807
G3666755	g3666755	469	808
G3728883	g3728883	470	809
G4132495	g4132495	471	810
AI011448	g4133423	472	811
AI230746	g3814633	473	812
AW253370	g3104091	474	813
AA965106	g3138598	475	814
AI009609	g4133075	476	815

BG372547	g3019278	477	816
G4135366	g4135366	478	817
D50306	Slc15a1	479	818
D30035	Prdx1	480	819
			820
M63837	Pdgfra	481	821
J02749	Acaa	482	822
			823
X05341	Acaa2	483	824
M22631	Pcca	484	825
L11276	Acadl	485	554
			555
			556
D16479	Hadhb	486	826
NM_017005	Fh	487	827
NM_012891	Acadv1	488	828
AF160978	Ly68	489	829
U40652	Ptpn	490	830
X68101	trg	491	831
NM_022398	LOC64201	492	832
NM_019274	Colq	493	833
NM_024360	Hes1	494	834
AF034577	Pdk4	495	835
AF139830	Igfbp-5	496	836
AB047541	Idh3a	497	837
NM_022503	Cox7a3	498	838
D10041	Fac16	499	839
AB028626	Rasa3	500	840
AJ245619	Ctl1	501	841
NM_022540	Prdx3	502	842
NM_012817	Igfbp5	503	843

NM_031032	Gmfb	504	844
NM_032614	Txn12	505	845
NM_019147	Jag1	506	846
NM_012966	Hspe1	507	847
M22030	ETF	508	848
X61106	Pgy4	509	849
NM_012839	Cycs	510	850
AB047540	IDH3B	511	851
NM_022395	Pmpcb	512	852
AJ277747	Masp2	513	853
NM_024392	Hsd17b4	514	854
NM_031511	Igf2	515	855
NM_033349	Hagh	516	856
NM_031510	Idh1	517	857
NM_017267	Timm44	518	858
D50664	Slc15a1	519	859
NM_012985	Ndufa5	520	860
NM_031645	Ramp1	521	861
NM_024139	Chp	522	862
AJ271158	LOC171069	523	863
AF150082	Timm8a	524	864
NM_031354	Vdac2	525	865
NM_017306	Dci	149	204
NM_022594	Ech1	150	205
NM_017092	Tyro3	526	866
AB032178	Cox17	527	867
X56228	Tst	528	868
NM_032615	Mir16	529	869
X05634	Sod1	530	870
			871
			872

AJ245707	Hpcl2	531	873
J03621	Suc1g1	532	874
NM_019187	Coq3	533	875
NM_024001	RPT	534	876
NM_019278	Resp18	535	877
X97831	Slc25a20	536	878
NM_017283	Psma6	537	879
NM_031821	Snk	538	880
AF095449	Hadhsc	539	881
M89902	Bdh	540	882
D00729	D00729	151	206
AB041723	Pdcd8	541	883
AF285103	Psmb7	542	884
NM_031851	Phb	543	885
NM_031350	Pex3	544	886
NM_024386	Hmgcl	545	887
L14684	EF-G	546	888
U88295	Cpt2	547	889 890 891
AF239219	Slc21a11	548	892
M64780	Agm	549	893
AJ007704	Mlycd	550	894

5 Mapping the 355 Rat Probes (SEQ ID NOs: 551-894, 155, 157, 164, 171, 178, 179, 185, 188, 204-206) to Mouse 3T3L1 Cells in Culture: Since the 3T3L1 is a mouse cell line, the 355 EWAT probes (SEQ ID NOs: 551-894, 155, 157, 164, 171, 178, 179, 185, 188, 204-206) from rat were mapped to mouse homologs. The mapped mouse probes were then checked in the 3T3L1 PPAR γ experiments (as described in Example 3) for regulation. There were 74 probes corresponding to 57 genes which were regulated with magnitude of log(ratio) greater than 0.2 (and P-value of regulation less than 1% in

more than 3 experiments) in response to a PPAR γ agonist or partial agonist. These 57 genes are useful in the practice of the present invention as a toxicity-related population of genes. The nucleotide sequence identification numbers of these 74 probes are identified in Table 7, (SEQ ID NOs: 950-1019, 863, 93, 94, 97). These 74 probes (SEQ ID NOs: 950-1019, 863, 93, 94, 97) corresponded to 57 different genes. The nucleotide sequence identification numbers of these 57 genes identified in Table 7, (SEQ ID NOs: 895-949, 42, 45).

Table 7. PPAR γ _3T3L1_Toxicity_HeartWeight_Probe_74 (Species: Mouse Cell Line)

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
AK003953	Tst	895	950
AK013511	Ndufv2	896	951
AK004125	1110036H20Rik	897	952
AK005084	Ndufa4	898	953
AF412297	Ghitm	899	954
NM_026179	1300003D03Rik	900	955
AK007415	1810010A06Rik	901	956
NM_025384	1110003P16Rik	902	957
AK008511	Usmg5	903	863
AK018763	Agt	904	958
BC004045	LOC212442	905	959
AK005067	Chp-pending	906	960
AB047323	COX17	907	961
AK002483	0610010I20Rik	908	962
AK004390	1110067B02Rik	909	963
NM_026614	2900002J19Rik	910	964
AK008267	1810055D05Rik	911	965
AK009374	2310016A09Rik	912	966
AK003283	Mrpl13	913	967
NM_011058	Pdgfra	914	968

AK002593	Cox7b	915	969
AK005080	Suc1g1	916	970
AK002889	0610041L09Rik	917	971
BC005585	LOC231086	918	972
NM_020520	Slc25a20	919	973
AK002320	0610008C08Rik	920	974
BG172638	LOC218885	921	975
BC005792	Pte1	922	976
AK003975	1500004O06Rik	923	977
			978
NM_021532	Thyex3-pending	924	979
AK009364	1810015H18Rik	925	980
AK002452	1110008F13Rik	926	981
BC004020	BC004020	927	982
BB004706	MGC37634	928	983
NM_013898	Timm8a	929	984
AK004827	0610011D08Rik	930	985
AK004924	Nudt7	931	986
AK003393	Idh3a	932	987
AJ250489	Ramp1	933	988
X01756	Cycs	934	989
BC009134	AA959601	935	990
AI648018	2610207I16Rik	936	991
			992
			993
AJ131522	Mlycd	937	994
AF278699	Angptl4	938	995
			996
			997
NM_013743	Pdk4	42	93
			94

			998
Z71189	Acadvl	939	999 1000 1001
AF030343	Ech1	940	1002
D13664	Osf2-pending	941	1003
D50834	Cyp4b1	942	1004
L12447	Igfbp5	45	97
M93275	Adfp	943	1005 1006 1007
M96163	Snk	944	1008
U07159	Acadm	945	1009 1010 1011
U21489	Acadl	946	1012 1013 1014
U37501	Lama5	947	1015
X70398	D0H4S114	948	1016
X89998	Hsd17b4	949	1017 1018 1019

Toxicity values were calculated from the expression pattern of the 74 probes (SEQ ID NOs: 950-1019, 863, 93, 94, 97) of the toxicity-related population of genes in the following manner. The gene expression profile induced by rosiglitazone (used at an effective concentration of 600 nM) was used as template, and a scale factor S of a given treatment was determined to minimize the following X^2 :

$$\chi^2 = \sum_{i=1}^{74} (S \cdot R_i - X_i)^2 / (\sigma_{R_i}^2 + \sigma_{X_i}^2)$$

where R_i stands for the log(ratio) of the 74 probes whose expression was affected by the high dose of rosiglitazone, σ_{R_i} is the error of R_i , X_i stands for the log(ratio) of the 74 probes (SEQ ID NOs: 950-1019, 863, 93, 94, 97) from that treatment, and σ_{X_i} is the error of X_i . The scale factor S is defined as the toxicity value for that treatment.

To determine whether the toxicity values, calculated in the foregoing manner, correlated with an increase in heart weight *in vivo*, heart weights were plotted directly against the calculated toxicity values for 10 full or partial agonists of PPAR γ that were tested both *in vivo* in rat, and *in vitro* in 3T3L1 cell lines. The data used was obtained from administration of the highest dosage of each of the 10 compounds. The calculated toxicity values for 9 of the 10 compounds correlated highly with the *in vivo* heart weights (correlation 0.8, P-value = 1.8×10^{-3}). The fact that the calculated toxicity value for one of the 10 compounds did not correlate highly with the *in vivo* heart weight was probably because the dosage of this compound, *in vivo*, was relatively low (30 milligrams per kilogram body weight) compared to the dosage of the other nine compounds (>100 milligrams per kilogram body weight).

Thus, the 3T3L1 cell line is useful in the practice of the present invention to obtain gene expression data that correlates with an undesirable increase in heart weight caused by a PPAR γ agonist or antagonist.

Early Heart Weight Biomarkers in EWAT: EWAT responded to treatment with a PPAR γ agonist, or partial agonist, much more strongly than heart tissues. Therefore EWAT was a sensitive tissue in terms of magnitude of response. The 355 probes (SEQ ID NOs: 551-894, 155, 157, 164, 171, 178, 179, 185, 188, 204-206) corresponding to the toxicity-related population of 343 genes (SEQ ID NOs: 219-550, 104, 105, 112, 119, 126, 127, 133, 136, 149-151), described in this Example, were further analyzed to identify a sub-population of genes that are useful as early biomarkers for the onset of the adverse effect of heart weight increase due to administration of a PPAR γ agonist or partial agonist.

The 355 rat EWAT probes (SEQ ID NOs: 551-894, 155, 157, 164, 171, 178, 179, 185, 188, 204-206) were projected to the "747 tissue experiment" by homolog mapping,

and then selecting the subset of PPAR γ regulated genes from fat tissues. 46 mouse homologs were regulated in the one day and 2 day treatments. These 46 genes are useful in the practice of the present invention as a toxicity-related gene population. The nucleotide sequences of the 67 probes that hybridized to the 46 genes, identified in Table 8, (SEQ ID NOs: 1036-1057, 951, 955, 957, 863, 959, 960, 63, 962, 966, 971-974, 980, 981, 984, 987, 989, 991-996, 93, 94, 998-1001, 97, 1004-1014, 1017-1019), are set forth in the SEQUENCE LISTING. The nucleotide sequences of the corresponding 46 genes identified in Table 8, (SEQ ID NOs: 1020-1035, 896, 900, 902, 903, 905, 906, 13, 908, 912, 917-920, 925, 926, 929, 932, 934, 936-939, 42, 942-946, 45, 949), are set forth in the SEQUENCE LISTING. Among the 46 genes (SEQ ID NOs: 1020-1035, 896, 900, 902, 903, 905, 906, 13, 908, 912, 917-920, 925, 926, 929, 932, 934, 936-939, 42, 942-946, 45, 949) regulated in the mouse fat tissues, 44 probes overlapped with the 74 3T3L1 probes (SEQ ID NOs: 950-1019, 863, 93, 94, 97).

Table 8. PPAR γ _Mouse_eWAT_Toxicity_HeartWeight_EarlyProbe_67 (Species: Mouse)

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
AK010479	2410012P20Rik	1020	1036
AK013511	Ndufv2	896	951
NM_026179	1300003D03Rik	900	955
NM_008303	Hspe1	1021	1037
NM_025384	1110003P16Rik	902	957
AK008511	Usmg5	903	863
NM_011192	Psme3	1022	1038
BC004045	LOC212442	905	959
AK018125	Gfm	1023	1039
AK005067	Chp-pending	906	960
AK004867	1300002P22Rik	13	63
AF058955	Sucla2	1024	1040
AK002483	0610010I20Rik	908	962
NM_019975	Hpcl-pending	1025	1041

AK009575	Bdh	1026	1042
AK008788	2610003B19Rik	1027	1043
AK009374	2310016A09Rik	912	966
AK013955	3110001K13Rik	1028	1044
AK003325	1110002N22Rik	1029	1045
AK002889	0610041L09Rik	917	971
BC005585	LOC231086	918	972
NM_020520	Slc25a20	919	973
NM_019961	Pex3	1030	1046
NM_026494	AI413471	1031	1047
AK002320	0610008C08Rik	920	974
AK009364	1810015H18Rik	925	980
AK002452	1110008F13Rik	926	981
NM_013898	Timm8a	929	984
AK015530	4930469P12Rik	1032	1048
AK003393	Idh3a	932	987
AI195543	MGC29978	1033	1049
X01756	Cycs	934	989
AI648018	2610207I16Rik	936	991 992 993
Z14050	Dci	1034	1050
AJ131522	Mlycd	937	994 1051
AF278699	Angptl4	938	995 996
NM_013743	Pdk4	42	93 998 94
Z71189	Acadvl	939	999 1000

			1001
D50834	Cyp4b1	942	1052 1053 1004
L12447	Igfbp5	45	1054 97 1055
M93275	Adfp	943	1005 1006 1007
M96163	Snk	944	1008
U01163	Cpt2	1035	1056 1057
U07159	Acadm	945	1011 1010 1009
U21489	Acadl	946	1012 1013 1014
X89998	Hsd17b4	949	1018 1017 1019

Plasma Volume Expansion Biomarkers in EWAT and 3T3L1 Cells: Using the same procedure that is described in this Example in the section entitled "Measuring the Toxic Effects of PPAR γ Agonists and PPAR γ Partial Agonists in Rats" for identifying heart weight biomarkers in EWAT, 271 probes were identified in EWAT whose expression was affected by a PPAR γ full agonist or partial agonist, and that correlated with plasma volume expansion (PVE). The nucleotide sequences of the 271 probes identified in Table 9, (SEQ ID NOs: 1239-1428, 558, 561, 158, 565, 574, 576, 578, 585, 592, 597, 600, 609, 612, 613, 617, 163, 625, 641-643, 646, 647, 655-657, 661, 666, 171,

681, 697, 700, 706, 707, 712, 720, 727, 740, 745, 748, 749, 755-757, 762, 766, 767, 769-771, 773, 778, 780, 786, 789, 794, 800, 803, 804, 188, 189, 191, 813, 814, 822, 823, 556, 828, 831, 832, 836, 840, 844, 864, 871, 876, 878, 883, 884, 889-891), are set forth in the SEQUENCE LISTING. 259 genes correspond to the 271 probes (SEQ ID NOs: 1239-1428, 558, 561, 158, 565, 574, 576, 578, 585, 592, 597, 600, 609, 612, 613, 617, 163, 625, 641-643, 646, 647, 655-657, 661, 666, 171, 681, 697, 700, 706, 707, 712, 720, 727, 740, 745, 748, 749, 755-757, 762, 766, 767, 769-771, 773, 778, 780, 786, 789, 794, 800, 803, 804, 188, 189, 191, 813, 814, 822, 823, 556, 828, 831, 832, 836, 840, 844, 864, 871, 876, 878, 883, 884, 889-891). The nucleotide sequences of these 259 genes as identified in Table 9 (SEQ ID NOs: 1058-1238, 222, 224, 106, 226, 235, 237, 239, 246, 253, 258, 261, 270, 273, 274, 278, 111, 286, 302-304, 307, 308, 316-318, 322, 327, 119, 342, 358, 361, 367, 368, 373, 381, 388, 401, 406, 409, 410, 416-418, 423, 427, 428, 430-432, 434, 439, 441, 447, 450, 455, 461, 464, 465, 136, 137, 139, 474, 475, 482, 485, 488, 491, 492, 496, 500, 504, 524, 530, 534, 536, 541, 542, 547), are set forth in the SEQUENCE LISTING.

Table 9. PPAR γ _Rat_eWAT_Toxicity_PVE_Probe_271 (Species: Rat)

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
J02752	RATACOA1	1058	1239 1240
J05030	Acads	1059	1241 1242
K03249	Ehhadh	222	558
M17701	Gapd	1060	1243 1244 1245
M29853	Cyp4b1	224	561
AA875107	AA875107	1061	1246
U39208	CYP4F6	1062	1247
U68544	cyclophilin D	1063	1248
Y09333	Mte1	106	158

AI170251	g3710291	226	565
AW523642	g4133650	1064	1249
701221122H1	701221122H1	1065	1250
BF288270	g2937947	1066	1251
BF415385	g3711895	1067	1252
G3332690	g3332690	1068	1253
G3705868	g3705868	1069	1254
BE111773	g2938661	1070	1255
G3708088	g3708088	1071	1256
G2936894	g2936894	1072	1257
AW918940	g4134740	1073	1258
AI113016	g3512965	235	574
G3103828	g3103828	237	576
G3816318	g3816318	1074	1259
AI408705	g2863227	239	578
G3710568	g3710568	1075	1260
G979671	g979671	1076	1261
BF420654	g3227012	1077	1262
G3189034	g3189034	246	585
G2948676	g2948676	1078	1263
G2939411	g2939411	1079	1264
AI144876	Ass	253	592
G2948912	g2948912	1080	1265
AI411031	g3709121	258	597
G2862965	g2862965	261	600
G4132595	g4132595	1081	1266
G3812213	g3812213	1082	1267
BG373361	g3333793	1083	1268
G2672793	g2672793	1084	1269
G3292487	g3292487	1085	1270
G3226140	g3226140	1086	1271

G3727666	g3727666	1087	1272
G3730290	g3730290	1088	1273
BE109153	g3638407	1089	1274
BF560807	g3187199	270	609
G3071873	g3071873	273	612
AA799476	g2862431	274	613
G3708991	g3708991	1090	1275
AI411212	g3710380	278	617
BG376920	g2864026	1091	1276
G3187055	g3187055	1092	1277
701221494H1	701221494H1	1093	1278
G3396562	g3396562	1094	1279
AI138016	g3638793	1095	1280
G3709353	g3709353	1096	1281
G3816414	g3816414	1097	1282
AA848702	g2936242	1098	1283
G3638603	g3638603	111	163
G3813131	g3813131	286	625
G3102919	g3102919	1099	1284
AI013919	g4133944	1100	1285
AI104605	g4134272	1101	1286
BG378613	g3103045	1102	1287
BG381472	g3726883	1103	1288
G2979890	g2979890	1104	1289
G2937670	g2937670	1105	1290
AA850195	g2937735	1106	1291
g3706559	g3706559	1107	1292
AA800179	g2863134	1108	1293
AI230578	g3814465	1109	1294
BE109153	g3637263	1110	1295
g3636884	g3636884	302	641

AA848951	g2936491	1111	1296
BF284475	g3711260	303	642
AA799707	g4131430	1112	1297
AA894090	g3020969	304	643
BE113034	g3815452	307	646
G3397918	g3397918	1113	1298
G3828291	g3828291	1114	1299
G3137782	g3137782	308	647
G3728910	g3728910	1115	1300
AI229639	g3813526	1116	1301
AI170808	g3710848	316	655
AA963282	g3136774	1117	1302
G3727129	g3727129	317	656
AW528443	g4136134	318	657
G3333614	g3333614	1118	1303
BE110615	g3226627	1119	1304
G3512087	g3512087	1120	1305
BF556962	g3708808	322	661
G3712131	g3712131	1121	1306
AW916776	g3667631	1122	1307
G2889306	g2889306	1123	1308
G3398898	g3398898	1124	1309
AA963069	g3136561	327	666
AI071994	g3398188	1125	1310
AA858867	g2948218	1126	1311
AI170067	g3710107	119	171
AI412011	g3247895	1127	1312
g3511496	g3511496	1128	1313
G3710033	g3710033	1129	1314
BE109401	g3247351	1130	1315
G3019865	g3019865	1131	1316

G3813191	g3813191	1132	1317
G3815059	g3815059	1133	1318
G4132386	g4132386	1134	1319
g3398472	g3398472	1135	1320
AA819658	g2888922	1136	1321
AA998205	g3188856	342	681
AA924580	g3071716	1137	1322
G980031	g980031	1138	1323
700691760H1	700691760H1	1139	1324
AI234620	g3828126	1140	1325
701216507H1	701216507H1	358	697
BG380734	g2938750	1141	1326
BG377008	g2863410	361	700
AW918113	g3291307	1142	1327
G3730272	g3730272	1143	1328
AI058968	g3332745	367	706
701349156H1	701349156H1	368	707
700692031H1	700692031H1	1144	1329
G980946	g980946	1145	1330
701219843H1	701219843H1	1146	1331
AI577393	g980620	1147	1332
701350827H1	701350827H1	1148	1333
700506509H1	700506509H1	1149	1334
700508607H1	700508607H1	373	712
600512417R1	600512417R1	381	720
G4134738	g4134738	388	727
600521579R1	600521579R1	1150	1335
600519254R1	600519254R1	1151	1336
G3225638	g3225638	401	740
600518885R1	600518885R1	1152	1337
600524228R1	600524228R1	1153	1338

AI010433	CdtwI	406	745
G3710810	g3710810	1154	1339
BG381033	g4131620	409	748
600512426R1	600512426R1	410	749
AW915824	600510363R1	1155	1340
600518233R1	600518233R1	1156	1341
AI599296	g3711488	1157	1342
G3103745	g3103745	1158	1343
G4134262	g4134262	416	755
AI009817	g3223649	1159	1344
600523104R1	600523104R1	417	756
600520906R1	600520906R1	418	757
AI101492	g4134011	1160	1345
AA892500	g3019379	1161	1346
AI411374	g3709749	1162	1347
G3815486	g3815486	423	762
600512215R1	600512215R1	1163	1348
BG376528	g3707272	1164	1349
600519560R1	600519560R1	1165	1350
AA800476	g2863431	1166	1351
G3104296	g3104296	427	766
600514084R1	600514084R1	428	767
BF394796	600515077R1	1167	1352
600508574R1	600508574R1	430	769
600516676R1	600516676R1	1168	1353
G3036598	g3036598	1169	1354
AA875107	g2980055	431	770
AI104528	g3708870	432	771
AA799741	g2862696	1170	1355
AJ005161	EF-Ts	1171	1356
G3104097	g3104097	1172	1357

AI171656	g3711696	434	773
700506775H1	700506775H1	1173	1358
AI104348	g3708719	439	778
AI045456	g3292275	1174	1359
G3831232	g3831232	441	780
BE349717	g3020180	1175	1360
G976906	g976906	1176	1361
BE101298	g3334069	1177	1362
G3019879	g3019879	447	786
g3018118	g3018118	1178	1363
BG381624	g3018621	450	789
700688496H1	700688496H1	1179	1364
AI145756	g3667555	1180	1365
BF282282	g3730624	1181	1366
AA801227	g4131587	1182	1367
AA800782	g4131537	455	794
BF413204	g3726768	1183	1368
AI071674	g3397889	1184	1369
AA859467	g2948987	1185	1370
G4135910	g4135910	461	800
BF282978	g3019668	1186	1371
BF394796	g3332553	1187	1372
G978793	g978793	464	803
G3707669	g3707669	465	804
G3709693	g3709693	1188	1373
AI231798	g3815678	1189	1374
AI227820	MgII	136	188
G3813792	g3813792	1190	1375
g3104887	g3104887	1191	1376
AA892864	MgII	137	189
G3222645	g3222645	1192	1377

G977669	g977669	139	191
AW253370	g3104091	474	813
AA965106	g3138598	475	814
G3812897	g3812897	1193	1378
AW913838	g3222273	1194	1379
D10952	Cox5b	1195	1380
J02749	Acaa	482	822 823
L11276	Acadl	485	556
D16236	Cdc25a	1196	1381
NM_012891	Acadvl	488	828
AF061266	Trrp1	1197	1382
X68101	trg	491	831
NM_022398	LOC64201	492	832
NM_022182	Fgf7	1198	1383
NM_013168	Hmbs	1199	1384
AF139830	Igfbp-5	496	836
AB028626	Rasa3	500	840
M29341	Gapd	1200	1243 1385
AW917188	Dpyd	1201	1386 1387
AF044574	Decr2	1202	1388
M96374	Nrxn1	1203	1389
AF170918	Aldh9a1	1204	1390 1391
NM_031032	Gmfb	504	844
NM_017280	Psma3	1205	1392
NM_012569	Gls	1206	1393
AB052846	Sc5d	1207	1394
NM_017020	Il6r	1208	1395

NM_021767	Nrxn1	1209	1396
L35921	Gng8	1210	1397
NM_017183	Il8rb	1211	1398
AB006614	Ucp3	1212	1399 1400 1401
NM_023023	Crmp5	1213	1402
NM_017321	Ratireb	1214	1403
AF150091	Timm10	1215	1404
NM_019352	Timm23	1216	1405
AF019109	Sort1	1217	1406
NM_031062	Mvd	1218	1407
AF026554	Slc5a6	1219	1408
J05446	Gys2	1220	1409
NM_022541	Ddp2	1221	1410
NM_031151	Mor1	1222	1411
AF021854	Pecr	1223	1412
NM_017256	Tgfbr3	1224	1413
NM_024398	Aco2	1225	1414
NM_023964	Gapds	1226	1415
D28560	Enpp2	1227	1416
AF150082	Timm8a	524	864
NM_031527	Ppp1ca	1228	1417
X54510	Atp5j	1229	1418
NM_024148	Apex	1230	1419
X05634	Sod1	530	871
NM_022500	Ftl1	1231	1420
NM_017006	G6pd	1232	1421
NM_024001	RPT	534	876
X97831	Slc25a20	536	878
D88891	Bach	1233	1422

AB041723	Pdcd8	541	883
AF285103	Psmb7	542	884
AY034383	Dlc2	1234	1423
U88295	Cpt2	547	889 890 891
NM_017177	Chetk	1235	1424
U00926	Atp5d	1236	1425
J04044	Alas1	1237	1426 1427
AF239045	Kidins220	1238	1428

Mapping these 271 EWAT probes (SEQ ID NOs: 1239-1428, 558, 561, 158, 565, 574, 576, 578, 585, 592, 597, 600, 609, 612, 613, 617, 163, 625, 641-643, 646, 647, 655-657, 661, 666, 171, 681, 697, 700, 706, 707, 712, 720, 727, 740, 745, 748, 749, 755-757, 762, 766, 767, 769-771, 773, 778, 780, 786, 789, 794, 800, 803, 804, 188, 189, 191, 813, 814, 822, 823, 556, 828, 831, 832, 836, 840, 844, 864, 871, 876, 878, 883, 884, 889-891) to mice yielded 44 probes that were also regulated by PPAR γ agonists in the mouse 3T3L1 cell line. The nucleotide sequences of the 44 probes identified in Table 10, (SEQ ID NOs: 1449-1471, 952, 956, 957, 963, 975, 976, 981, 983, 984, 986, 990, 999-1001, 1004-1007, 1012-1014), are set forth in the SEQUENCE LISTING. The nucleotide sequences of the corresponding 35 genes identified in Table 10, (SEQ ID NOs: 1429-1448, 897, 901, 902, 919, 921, 922, 926, 928, 929, 931, 935, 939, 942, 943, 946), are set forth in the SEQUENCE LISTING.

15 Table 10. PPAR γ _3T3L1_Toxicity_PVE_Probe_44 (Species: Mouse Cell Line)

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
BC004645	Aco2	1429	1449 1450
AK004125	1110036H20Rik	897	952
AK007415	1810010A06Rik	901	956

AK007651	Ubqln1	1430	1451
NM_025384	1110003P16Rik	902	957
NM_015744	Enpp2	1431	1452
NM_019993	Aldh9a1	1432	1453
BC011289	6720463E02Rik	1433	1454
AK004193	1110046O21Rik	1434	1455
AK004954	1300010A20Rik	1435	1456
AK007497	1810014L12Rik	1436	1457
NM_024207	1110021N07Rik	1437	1458
AK004634	Gng3lg	1438	1459
AK008088	Timm13a	1439	1460
NM_020520	Slc25a20	919	973
AJ309922	Mvd	1440	1461
BG172638	LOC218885	921	975
BC005792	Pte1	922	976
NM_016897	Timm23	1441	1462
AK002452	1110008F13Rik	926	981
BC002251	AI480570	1442	1463
BB004706	MGC37634	928	983
NM_007658	Cdc25a	1443	1464
NM_013898	Timm8a	929	984
AK004924	Nudt7	931	986
BC009134	AA959601	935	990
Z71189	Acadv1	939	999 1000 1001
AF006688	Acox1	1444	1465 1466 1467
D50834	Cyp4b1	942	1004
M16229	Mor1	1445	1468

M93275	Adfp	943	1005 1006 1007
U21489	Acadl	946	1012 1013 1014
X53802	Il6ra	1446	1469
AB016248	Sc5d	1447	1470
NM_008008	Fgf7	1448	1471

It is noteworthy that the heart weight and PVE toxicity values from the 3T3L1 model system were highly correlated with the classifier values as described in Example 3. Therefore, in this example, using the 3T3L1 system, only the toxicity value or the classifier need be calculated for each compound.

EXAMPLE 3

This Example describes the identification of a classifier population of genes that is useful for classifying candidate agents as being more like a known agonist of PPAR γ , or as being more like a known partial agonist of PPAR γ .

The gene expression profile of 26 compounds at high dosage (30 x EC₅₀) in 3T3L1 adipocyte cell line were measured using a Rosetta mouse 25K DNA Microarray. The overall experiment was conducted in three phases (*i.e.*, in three separate experiments conducted at three different times) as shown in Table 11 below. Three replicates were done for each of the tested compounds in each phase of the experiment.

The gene expression measurement levels from the following compound treatments were used as the training set: PPAR γ partial agonists: 2-(3-{[3-(4-chlorobenzoyl)-2-methyl-6-(trifluoromethoxy)-1*H*-indol-1-yl]methyl}phenoxy)-3-methylbutanoate; (2*R*)-2-(4-chloro-3-{[3-(6-methoxy-1,2-benzisoxazol-3-yl)-2-methyl-6-(trifluoromethoxy)-1*H*-indol-1-yl]methyl}phenoxy)propanoate; (2*S*)-2-(4-chloro-3-{[1-(6-chloro-1,2-benzisoxazol-3-yl)-2-methyl-5-(trifluoromethoxy)-1*H*-indol-3-yl]oxy}phenoxy)propanoic acid; and (2*R*)-2-(2-chloro-5-{[3-(4-chlorobenzoyl)-2-methyl-6-(trifluoromethoxy)-1*H*-indol-1-yl]methyl}phenoxy)propanoic acid; and PPAR γ

agonists: 5-(4-{2-[methyl(pyridin-2-yl)amino]ethoxy}benzyl)-1,3-thiazolidine-2,4-dione, and 5-{4-[2-hydroxy-2-(5-methyl-2-phenyl-1,3-oxazol-4-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione.

The other PPAR γ agonist, and partial agonist, compounds were used in testing the classifier population of genes. The following dosages were used where indicated by a * 0.540 μ M in Phase 1, 0.600 μ M in Phases 2 and 3; and where indicated by a ** 6.3 μ M in Phase 2, 6.324 μ M in Phase 3. The PPAR α agonist was included as a control.

Table 11.

Phase 1	Phase 2	Phase 3	Compounds	Dosage (μ M)
	X	X	PPAR α agonist	10.0
	X		Partial agonist 2	0.030
X			Partial agonist 3	0.300
	X	X	Partial agonist 4	**
	X		Partial agonist 2-(3-{[3-(4-chlorobenzoyl)-2-methyl-6-(trifluoromethoxy)-1 <i>H</i> -indol-1-yl]methyl}phenoxy)-3-methylbutanoate	3.0
X	X	X	Partial agonist (2 <i>R</i>)-2-(4-chloro-3-{[3-(6-methoxy-1,2-benzisoxazol-3-yl)-2-methyl-6-(trifluoromethoxy)-1 <i>H</i> -indol-1-yl]methyl}phenoxy)propanoate	*
	X		Partial agonist 5	0.3
	X		Partial agonist 6	10.0
X			Partial agonist (2 <i>S</i>)-2-(4-chloro-3-{[1-(6-chloro-1,2-benzisoxazol-3-yl)-2-methyl-5-(trifluoromethoxy)-1 <i>H</i> -indol-3-yl]oxy}phenoxy)propanoic acid	0.12
	X		Partial agonist 7	1.4
	X		Partial agonist 8	0.1
		X	Partial agonist 9	0.158
		X	Partial agonist 10	0.285

Phase 1	Phase 2	Phase 3	Compounds	Dosage (μM)
X			Partial agonist (2 <i>R</i>)-2-(2-chloro-5-{[3-(4-chlorobenzoyl)-2-methyl-6-(trifluoromethoxy)-1 <i>H</i> -indol-1-yl]methyl}phenoxy)propanoic acid	0.054
	X	X	Partial agonist 11	1.1
		X	Partial agonist 12	0.221
	X	X	Partial agonist 13	1.8
		X	Partial agonist 14	0.126
		X	Partial agonist 15	0.2
		X	Partial agonist 16	16.032
		X	Partial agonist 17	1.075
X		X	Agonist 1	3.870
X			Agonist 2	0.006
	X		Agonist 3	1.5
X	X	X	Agonist 5-(4-{2-[methyl(pyridin-2-yl)amino]ethoxy}benzyl)-1,3-thiazolidine-2,4-dione)	*
X			Agonist (5-{4-[2-hydroxy-2-(5-methyl-2-phenyl-1,3-oxazol-4-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione)	0.027

The three replicate gene expression profiles within each phase of the experiment were first combined based on the error-weighted average. Expression profiles of two PPAR γ full agonists, and four PPAR γ partial agonists (in Phase 1) were chosen for classifier training, and were divided into the following two groups:

Group 1: two PPAR γ full agonists (5-(4-{2-[methyl(pyridin-2-yl)amino]ethoxy}benzyl)-1,3-thiazolidine-2,4-dione and 5-{4-[2-hydroxy-2-(5-methyl-2-phenyl-1,3-oxazol-4-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione)

Group 2: four PPAR γ partial agonists ((2*R*)-2-(2-chloro-5-{[3-(4-chlorobenzoyl)-2-methyl-6-(trifluoromethoxy)-1*H*-indol-1-yl]methyl}phenoxy)propanoic acid; (2*S*)-2-(4-chloro-3-{[1-(6-chloro-1,2-benzisoxazol-3-yl)-2-methyl-5-(trifluoromethoxy)-1*H*-indol-3-yl]oxy}phenoxy)propanoic acid; (2*S*)-2-(3-{[1-(4-methoxybenzoyl)-2-methyl-5-(trifluoromethoxy)-1*H*-indol-3-yl]methyl}phenoxy)propanoic acid; and (2*R*)-2-(4-chloro-3-{[3-(6-methoxy-1,2-benzisoxazol-3-yl)-2-methyl-6-(trifluoromethoxy)-1*H*-indol-1-yl]methyl}phenoxy)propanoate).

The expression profiles of the remaining compounds were used to test the classifier gene population.

Probes identified in the training gene set that had a pvalue of less than 0.1 in at least one of the above training compound expression profiles were selected. A total of 7,610 probes were selected. The Matlab function ANOVA1 (one-way analysis of variance) was used to calculate the pvalue (hereafter referred to as the ANOVA-pvalue) for the null hypothesis that the means of Group 1 and Group 2 are equal. Probes with an ANOVA-pvalue smaller than 1×10^{-7} and an absolute value of the average of logRatio in Group 1 greater than $\log_{10} 1.5$ (which is a value of 0.1761) were selected. The resulting 303 probes corresponded to 290 genes that were the classifier population that were PPAR γ agonist signature genes and that best distinguished partial PPAR γ agonists from full PPAR γ agonists.

The nucleotide sequences of the 303 probes identified in Table 12, (SEQ ID NOs: 1731-1996, 52, 951, 1450, 957, 1452, 1455, 65, 68, 69, 72, 75, 1457, 967, 1458, 970, 971, 974, 1462, 82, 977, 978, 982, 90, 989, 990, 215, 999-1001, 96, 1468, 1005, 1006, 218, 1014, 1018, 1019), are set forth in the SEQUENCE LISTING. The nucleotide sequences of the corresponding 290 genes identified in Table 12, (SEQ ID NOs: 1472-1730, 2, 896, 1429, 902, 1431, 15, 18, 19, 22, 25, 1436, 913, 1437, 916, 917, 920, 1441, 32, 923, 927, 39, 934, 935, 210, 939, 44, 1445, 943, 212, 946, 949), are set forth in the SEQUENCE LISTING.

Table 12. PPAR γ _3T3L1_Compound_Classifier_Probe_303 (Species: Mouse Cell Line)

Accession_number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
AK005615	1700001N19Rik	1472	1731

NM_007760	Crat	1473	1732
AK013984	3110003A17Rik	1474	1733
AW909114	MGC28611	2	52
AK003912	1110025G12Rik	1475	1734
AK013511	Ndufv2	896	951
AK009628	2310035C23Rik	1476	1735
NM_021704	Cxcl12	1477	1736
AK003232	Cbr3	1478	1737
BC002149	4633402C03Rik	1479	1738
AK011998	2610528M18Rik	1480	1739
AK009071	2310001K24Rik	1481	1740
AK016432	4931406C07Rik	1482	1741
AK017037	4930433D19Rik	1483	1742
BC004645	Aco2	1429	1450
NM_011677	Ung	1484	1743
AK013880	Nars	1485	1744
NM_010697	Ldb1	1486	1745
AK019322	2900029G13Rik	1487	1746
NM_011868	Peci	1488	1747
NM_011921	Aldh1a7	1489	1748
NM_025772	Dtnbp1	1490	1749
AK004338	1110061E11Rik	1491	1750
NM_011031	P4ha2	1492	1751
NM_007672	Cdr2	1493	1752
NM_015734	Col5a1	1494	1753
AK010791	2410131K14Rik	1495	1754
NM_011701	Vim	1496	1755
NM_011050	Pdcd4	1497	1756
NM_016861	Pdlim1	1498	1757
AK011193	2600013D04Rik	1499	1758
NM_020026	B3galt3	1500	1759

NM_008768	Orm1	1501	1760
AV367848	AA959574	1502	1761
AK005869	1700011111Rik	1503	1762
NM_008590	Mest	1504	1763
BI689765	AA617265	1505	1764
AK008764	2210021K23Rik	1506	1765
NM_025384	1110003P16Rik	902	957
NM_010634	Fabp5	1507	1766
AK012054	2610319K07Rik	1508	1767
NM_015744	Enpp2	1431	1452
AF294617	Pfkfb3	1509	1768
AV298518	AV298518	1510	1769
AK004987	Mkks	1511	1770
X15052	Ncam1	1512	1771
NM_007473	Aqp7	1513	1772
AK007902	1810059C13Rik	1514	1773
AK019783	4930564I24Rik	1515	1774
BC005552	Asns	1516	1775
NM_016762	Matn2	1517	1776
NM_007881	Drpla	1518	1777
AK009197	2310007D03Rik	1519	1778
AK013761	2900070E19Rik	1520	1779
NM_009320	Slc6a6	1521	1780
NM_008520	Ltbp3	1522	1781
AK004614	1200006I17Rik	1523	1782
NM_008638	Mthfd2	1524	1783
AK012758	1200014I03Rik	1525	1784
NM_011424	Ncor2	1526	1785
AK020007	5830411O09Rik	1527	1786
AV341581	6330577E15Rik	1528	1787
AK008165	2010009K05Rik	1529	1788

NM_032398	Plvap	1530	1789
NM_011693	Vcam1	1531	1790
BC003432	Etfa	1532	1791
AK005710	Slc25a19	1533	1792
NM_011641	Trp63	1534	1793
AK004743	Myo1c	1535	1794
NM_009149	Selel	1536	1795
NM_009058	Rgds	1537	1796
AK004759	1200014F01Rik	1538	1797
AK004153	1110038D17Rik	1539	1798
AK010185	2310075M15Rik	1540	1799
AK002769	0610037F22Rik	1541	1800
AK019459	Atp5f1	1542	1801
AF179996	Sept8	1543	1802
NM_011462	Spin	1544	1803
AK017610	2810011K15Rik	1545	1804
NM_021893	Pcdcl1g1	1546	1805
AK004193	1110046O21Rik	1434	1455
BC003988	Rbm5	1547	1806
AK009315	2310012G06Rik	15	65
AK021117	C030033M12Rik	1548	1807
AV378562	2410022M24Rik	1549	1808
NM_007945	Eps8	1550	1809
NM_008608	Mmp14	1551	1810
NM_013655	Cxcl12	1552	1811
AK003270	Tbgl	1553	1812
AK006810	2210018M03Rik	1554	1813
AK005515	1600021P15Rik	1555	1814
BB001681	MICAL-3	1556	1815
AK021325	D730003I15Rik	1557	1816
NM_011782	Adamts5	18	68

AW120656	MGC28924	1558	1817
AK002851	0610039N19Rik	1559	1818
NM_011598	Tlbp	1560	1819
AV075202	Acadvl	1561	1820
AK013448	2810487F15Rik	1562	1821
NM_019729	Usp8	1563	1822
NM_020578	Ehd3	19	69
BE947541	BE947541	1564	1823
AK017403	5430437E11Rik	1565	1824
AK004526	1810061M12Rik	1566	1825
AK004642	Lfng	1567	1826
NM_011766	Zfpm2	1568	1827
AK010506	Pbx4	1569	1828
BB113348	BB113348	1570	1829
AK019860	Agpt2	1571	1830
AK018466	8430436O14Rik	1572	1831
AK013157	2810425J22Rik	1573	1832
AK010891	2510002J07Rik	22	72
AK002480	0610010I13Rik	1574	1833
NM_008735	Nrip1	1575	1834
AK007896	Cdc42ep1	1576	1835
NM_015757	Pcdh13	1577	1836
AW476152	Adamts2	1578	1837
NM_007941	Epim	1579	1838
AK011976	Angptl2	1580	1839
AK007873	1810055P05Rik	1581	1840
AK004732	1200013A08Rik	25	75
NM_021528	C4st2-pending	1582	1841
AK009739	Klf15	1583	1842
AK014643	4733401N06Rik	1584	1843
AV221349	ri 3322401K10 PX00010E04 22	1585	1844

	95		
AK004659	Cfl2	1586	1845
AK007497	1810014L12Rik	1436	1457
AK004770	9130009D18Rik	1587	1846
NM_023294	2610020P18Rik	1588	1847
AK004670	1200009F10Rik	1589	1848
NM_023058	Pkmyt1-pending	1590	1849
BI101760	AW214504	1591	1850
AK011889	2610205H19Rik	1592	1851
NM_011812	Fbln5	1593	1852
NM_008216	Has2	1594	1853
AK003283	Mrpl13	913	967
NM_007705	Cirbp	1595	1854
NM_025892	1500031L02Rik	1596	1855
NM_024207	1110021N07Rik	1437	1458
AK002277	Igfbp7	1597	1856
NM_008564	Mcmd2	1598	1857
AV102233	AV102233	1599	1858
NM_008486	Anpep	1600	1859
BC002107	D5ErtD371e	1601	1860
NM_007970	Ezh1	1602	1861
AK002744	0610033L03Rik	1603	1862
AK017684	5730466C23Rik	1604	1863
AK003387	Ube2g2	1605	1864
AK002942	0610020I02Rik	1606	1865
NM_010225	Foxf2	1607	1866
AV077222	2810422B09Rik	1608	1867
AK007959	Klf3	1609	1868
AK021144	C030044C12Rik	1610	1869
BF160060	AV212693	1611	1870
NM_025910	1810047J07Rik	1612	1871

AV247986	Dysf	1613	1872
AK017918	5830411H19Rik	1614	1873
AK005080	Sucgl1	916	970
AW490567	Jag1	1615	1874
AV238629	AV238629	1616	1875
AK006128	Abcc3	1617	1876
AK002889	0610041L09Rik	917	971
AK018089	6230416A05Rik	1618	1877
NM_008810	Pdha1	1619	1878
NM_025626	3110001A13Rik	1620	1879
AF096898	D15Mit260	1621	1880
AK003535	1110007F12Rik	1622	1881
NM_023644	Mccc1	1623	1882
AK008125	2010005I16Rik	1624	1883
BC004702	Birc5	1625	1884
BE553640	1700084G18Rik	1626	1885
AJ276796	Cars	1627	1886
NM_019804	B4galt4	1628	1887
AK008255	2010015J01Rik	1629	1888
NM_011796	Capn10	1630	1889
AK004851	1300002F13Rik	1631	1890
NM_007620	Cbr1	1632	1891
AK010706	2410055N02Rik	1633	1892
AK008822	4933404O11Rik	1634	1893
NM_010918	Nktr	1635	1894
AK002320	0610008C08Rik	920	974
NM_009104	Rrm2	1636	1895
BC004801	LOC207933	1637	1896
AK009291	2310011D08Rik	1638	1897
NM_010422	Hexb	1639	1898
AK013062	2810410A03Rik	1640	1899

AK003556	2310075G14Rik	1641	1900
NM_016788	Tnk2	1642	1901
NM_007707	Cish3	1643	1902
NM_016897	Timm23	1441	1462
NM_016810	Gosr1	1644	1903
AK016659	4933405A16Rik	1645	1904
AK020118	6720429C22Rik	1646	1905
AK020182	7330412A13Rik	1647	1906
AK011182	2600010N21Rik	1648	1907
NM_009378	Thbd	1649	1908
AK007856	1810054D07Rik	1650	1909
NM_024223	Crip2	1651	1910
AK020048	6030408B16Rik	1652	1911
AK019002	1810004I06Rik	1653	1912
AK013740	6530401D17Rik	32	82
AK010344	2410002L19Rik	1654	1913
NM_011479	Sptlc2	1655	1914
AK003709	1110014L14Rik	1656	1915
NM_025809	1200003C23Rik	1657	1916
AK008679	2210008N01Rik	1658	1917
AK003975	1500004O06Rik	923	978 977
AK010747	2410089E03Rik	1659	1918
NM_026473	2310057H16Rik	1660	1919
NM_008910	Ppm1a	1661	1920
AK003621	1110012D08Rik	1662	1921
AK004432	1190001I08Rik	1663	1922
AK018500	2700038I16Rik	1664	1923
AK016881	4933424A20Rik	1665	1924
NM_026842	Ubqln1	1666	1925
BC004020	BC004020	927	982

AK002699	Ptk9l	1667	1926
NM_008841	Pik3r2	1668	1927
NM_016812	Banp	1669	1928
BC003261	Stk5	1670	1929
AK003995	1110030N17Rik	1671	1930
NM_007996	Fdx1	1672	1931
NM_013792	Naglu	1673	1932
AC002397	CD4,A-2,B,GNB3, C8, ISOT, TPI, B7, ENO2, DRPLA, U7snRNA, C10, PTPN6, BAP, C2F	1674	1933
NM_017370	Hp	1675	1934
AK010043	2310065E01Rik	1676	1935
BC003908	2310046B19Rik	1677	1936
NM_007609	Casp11	1678	1937
BE994229	Tcfcp2	1679	1938
NM_008055	Fzd4	1680	1939
AK003586	1110008K06Rik	1681	1940
AK013580	2900024C23Rik	1682	1941
BC004633	2410011G03Rik	1683	1942
AK009883	Atp5g1	1684	1943
AK010765	Bag4	1685	1944
AK002531	Sat	1686	1945
AK016103	4930553F04Rik	39	90
BC003766	Nfix	1687	1946
BC010825	1700112L09Rik	1688	1947
U03419	Colla1	1689	1948
U03715	Coll8a1	1690	1949
M20497	Fabp4	1691	1950
AA543477	Mgst1	1692	1951
Z38015	DM-PK	1693	1952

X01756	Cycs	934	989
L02331	Sult1a1	1694	1953
BC007148	Vps26	1695	1954
AF013262	Lum	1696	1955
BC009134	AA959601	935	990
BC008989	LOC217166	1697	1956
M13264	Fabp4	210	215
Z71189	Acadv1	939	1001 999 1000
AF007267	Pmm1	1698	1957
AF011450	Col15a1	1699	1958
AF057286	Epn2	1700	1959
D01093	Pcsk4	1701	1960
D86949	Plxna2	1702	1961
J04632	Gstm1	44	96
J04696	Gstm2	1703	1962
L02918	Col5a2	1704	1963
L57509	Ddr1	1705	1964
M16229	Mor1	1445	1468
M18194	Fn1	1706	1965
M32240	Pmp22	1707	1966 1967 1968
M93275	Adfp	943	1005 1006
U01841	Pparg	212	1969 1970 218
U03283	Cyp1b1	1708	1971 1972

U08020	Colla1	1709	1973
U14332	Il15	1710	1974
U21489	Acadl	946	1014
U43298	Lamb3	1711	1975
U58883	Sorbs1	1712	1976
			1977
U67187	Rgs2	1713	1978
U79550	Snai2	1714	1979
X04017	Sparc	1715	1980
X04367	Pdgfrb	1716	1981
			1982
X63535	Axl	1717	1983
X67469	Lrp1	1718	1984
X89998	Hsd17b4	949	1018
			1019
Y15163	Cited2	1719	1985
J03484	Lamc1	1720	1986
X04972	Sod2	1721	1987
X69620	Inhbb	1722	1988
AI314880	Tstap91a	1723	1989
AI746433	AI746433	1724	1990
U70139	Ccr4	1725	1991
AB023957	EIG180	1726	1992
NM_011513	Surf5	1727	1993
NM_010284	Ghr	1728	1994
AI448406	AI562151	1729	1995
AI449447	AI449447	1730	1996

The average of the logRatio of each of the 303 probes (SEQ ID NOs: 1731-1996, 52, 951, 1450, 957, 1452, 1455, 65, 68, 69, 72, 75, 1457, 967, 1458, 970, 971, 974, 1462, 82, 977, 978, 982, 90, 989, 990, 215, 999-1001, 96, 1468, 1005, 1006, 218, 1014, 1018,

1019) in Group 1 was calculated and served as the template. A classifier value for a PPAR γ agonist, or partial agonist, was calculated in the following manner. The value (expressed as a percentage) of the logRatio divided by the template logRatio for each of the 303 probes (SEQ ID NOs: 1731-1996, 52, 951, 1450, 957, 1452, 1455, 65, 68, 69, 72, 75, 1457, 967, 1458, 970, 971, 974, 1462, 82, 977, 978, 982, 90, 989, 990, 215, 999-1001, 96, 1468, 1005, 1006, 218, 1014, 1018, 1019) was calculated, and then the mean of the resulting 303 percentages was calculated. This mean value was the classifier value for the PPAR γ agonist, or partial agonist.

Table 13 below shows the classifier value for the compounds that were tested in Phase 3 of the 3T3L1 experiment.

TABLE 13.

Compound	Classifier Value
Agonist 1	0.881
Agonist 5-(4-{2-[methyl(pyridin-2-yl)amino]ethoxy}benzyl)-1,3-thiazolidine-2,4-dione)	0.850
Partial agonist 16	0.708
Partial agonist 15	0.651
Partial agonist 17	0.550
Partial agonist 4	0.473
Partial agonist 10	0.387
Partial agonist 13	0.363
Partial agonist 9	0.352
Partial agonist 12	0.350
Partial agonist (2 <i>R</i>)-2-(4-chloro-3-{[3-(6-methoxy-1,2-benzisoxazol-3-yl)-2-methyl-6-(trifluoromethoxy)-1 <i>H</i> -indol-1-yl]methyl}phenoxy)propanoate	0.341
Partial agonist 11	0.309
Partial agonist 14	0.302
PPAR α agonist	0.096

This classifier gene population is useful for ranking candidate partial agonists of PPAR γ and full agonists of PPAR γ relative to one or more known partial agonists of PPAR γ and one or more known full agonists of PPAR γ .

5

EXAMPLE 4

This Example describes the identification of a population of genes that yield an expression pattern that correlates with the stimulation of PPAR α receptors by an agent. This population of genes can be used, for example, to screen candidate PPAR γ agonists, or partial agonists, to identify those candidate agents that possess the undesirable property
10 of stimulating PPAR α receptors. This population of genes can also be used, for example, to identify PPAR α agonists, or PPAR α partial agonists.

Wild type mice, and mice that had been genetically modified to inactivate all copies of the gene encoding the PPAR α protein (called PPAR α knockout mice), were treated with PPAR α agonists. Genes whose expression was significantly affected in wild
15 type mice in response to the PPAR α agonists, but which was not significantly affected in PPAR α knockout mice, were identified. The resulting gene set was considered a PPAR α receptor-dependent signature gene set.

Two PPAR α agonists were orally administered to wild type mice (abbreviated as WT mice) and to PPAR α knockout mice (abbreviated as KO mice). The two compounds
20 were Fenofibrate (administered at a dosage of 200 milligrams per kilogram body weight), and [4-chloro-6-(2,3-xylylidino)-2-pyrimidinylthio]acetic acid (administered at a dosage of 30 milligrams per kilogram body weight). The PPAR α agonists were administered at day 1 and day 7. Three experimental conditions were tested for each PPAR α agonist:

WT control pool vs. WT treatment (hereafter WT vs. WT treatment)
25 KO control pool vs. KO treatment (hereafter KO vs. KO treatment)
WT treatment vs. KO treatment (hereafter WT treatment vs. KO treatment)

The hybrid ANOVA method described in Example 1 was used to calculate the ANOVA-pvalue and the average of logRatio of gene expression for each gene in each of the 12 experimental groups (*i.e.*, two drug treatments x two time points x three
30 conditions). Signature genes were identified that had an ANOVA-pvalue less than 0.01, and the absolute value of the average of logRatio greater than log₁₀1.5.

The union of the one day signature genes with the seven day signature genes for each of the two PPAR α agonist treatments under each of the three experimental conditions (WT vs. WT treatment; KO vs. KO treatment; WT treatment vs. KO treatment) was used to identify genes whose expression was significantly regulated in the WT vs. WT treatment, and WT treatment vs. KO treatment groups, but not in the KO vs. KO treatment group, for each of the two PPAR α agonist treatments. The genes that were common to the PPAR α agonist treatments were identified, thereby yielding a total of 978 probes as identified in Table 14, (SEQ ID NOs: 2796-3683, 1732, 1734, 53, 1740, 1449, 1450, 1747, 1748, 1037, 1759, 957, 1774, 60, 1780, 63, 1797, 962, 1808, 1041, 1809, 1817, 1818, 1820, 1824, 71, 72, 1833, 966, 1873, 970-973, 1879, 1046, 1047, 976, 1898, 1904, 80, 1910, 86, 1932, 1933, 1941, 1049, 989, 1953, 991-993, 1050, 1051, 994, 215, 216, 93, 94, 998-1001, 1465-1467, 1957, 1002, 214, 1962, 1005-1007, 1056, 1057, 1009-1014, 1974, 1975, 1977, 1979, 1016-1019, 1994, 101), corresponding to 870 unique genes as identified in Table 14, (SEQ ID NOs: 1997-2795, 1473, 1475, 3, 1481, 1429, 1488, 1489, 1021, 1500, 902, 1515, 10, 1521, 13, 1538, 908, 1549, 1025, 1550, 1558, 1559, 1561, 1565, 21, 22, 1574, 912, 1614, 916-919, 1620, 1030, 1031, 922, 1639, 1645, 30, 1651, 35, 1673, 1674, 1682, 1033, 934, 1694, 936, 1034, 937, 210, 42, 939, 1444, 1698, 940, 209, 1703, 943, 1035, 945, 1710, 946, 1711, 1712, 1714, 948, 949, 142, 1728, 49).

Table 14. PPAR α _3T3L1_Liver_Dependent_Regulation_Probe_978 (Species: Mouse Cell Line)

Accession number	Gene Name	Gene SEQ ID NO	Probe SEQ ID NO
AK005570	1600032L17Rik	1997	2796
NM_008298	Dnajl1	1998	2797
AW122190	AW122190	1999	2798
AK018646	9130022K13Rik	2000	2799
AK020256	9030616G12Rik	2001	2800
AK012001	2610306P15Rik	2002	2801
AV225723	AA408038	2003	2802
AK012577	2700087I09Rik	2004	2803

AK015314	0710001P09Rik	2005	2804
NM_019926	Mtm1	2006	2805
BE691027	BE691027	2007	2806
AK019063	2210408B16Rik	2008	2807
AK005808	1700010A17Rik	2009	2808
AV269843	MGC30495	2010	2809
AK014452	3830422K02Rik	2011	2810
NM_019723	Slc22a9	2012	2811
BC011492	9130020G10Rik	2013	2812
AI449628	AI449595	2014	2813
BC004092	Nd1-pending	2015	2814
NM_007760	Crat	1473	1732 2815
BF455494	BF455494	2016	2816
NM_021526	Pohl-pending	2017	2817
AK012370	Scd1	2018	2818
AK012685	2810007J24Rik	2019	2819
AK019713	4930529O08Rik	2020	2820
AK015561	4930472G13Rik	2021	2821
AK007857	1810054F20Rik	2022	2822
NM_028119	2610043A19Rik	2023	2823
AK015340	4930439B20Rik	2024	2824
NM_010139	Epha2	2025	2825
AK002693	Dgat2l1	2026	2826
AK016318	4930579F01Rik	2027	2827
AK013414	Sip1	2028	2828
NM_027288	2410030O07Rik	2029	2829
BC002151	1110056N09Rik	2030	2830
AK009210	2310007J06Rik	2031	2831
AV356694	AV356694	2032	2832
AK005622	Ins16	2033	2833

AK009377	2310016C08Rik	2034	2834
AK003912	1110025G12Rik	1475	1734
BB541540	Clcn2	2035	2835
NM_025558	1810044O22Rik	2036	2836
NM_008543	Madh7	3	53
NM_011596	Atp6v0a2	2037	2837
AF339106	Foxp2	2038	2838
AK003879	5730512J02Rik	2039	2839
NM_008878	Serpinf2	2040	2840
NM_018760	Slc4a4	2041	2841
NM_008129	Gclm	2042	2842
AK013628	2900040J22Rik	2043	2843
NM_008681	Ndr1	2044	2844
BF579112	AW121759	2045	2845
AK009071	2310001K24Rik	1481	1740
AK017628	5730438N18Rik	2046	2846
AK012088	Fac13	2047	2847
NM_026586	6720475J19Rik	2048	2848
NM_007930	Enc1	2049	2849
AK009134	Acyp2	2050	2850
BC004645	Aco2	1429	1449 1450 2851
AV278562	AV278562	2051	2852
AK018792	1520401O13Rik	2052	2853
AK010547	5730471K09Rik	2053	2854
NM_010237	Frk	2054	2855
AK014380	3321402G02Rik	2055	2856
NM_010001	Cyp2c37	2056	2857
NM_009794	Capn2	2057	2858
AK005616	1700001O02Rik	2058	2859

NM_027280	Nkd1	2059	2860
AK013597	2900026A02Rik	2060	2861
AK004307	Grhpr	2061	2862
NM_008253	Hmgb3	2062	2863
AK008360	Fcgrt	2063	2864
AK009343	2310014L03Rik	2064	2865
AV115239	AV115239	2065	2866
NM_008769	Otc	2066	2867
AK004782	Lgals8	2067	2868
AK011596	Trfr	2068	2869
NM_011868	Peci	1488	1747
AK006140	1700020A13Rik	2069	2870
W29450	AA410048	2070	2871
BC004728	BC004728	2071	2872
AL359935	LOC209798	2072	2873
BG970486	ri 1700025L02 ZX00037H10 1579	2073	2874
BC005759	Sec14l2	2074	2875
NM_011921	Aldh1a7	1489	1748
AK016187	4930562A09Rik	2075	2876
AK003420	1110004G24Rik	2076	2877
NM_023805	Slc38a3	2077	2878
AK018155	6330410P18Rik	2078	2879
AK004550	1200002M06Rik	2079	2880
AK013094	2810416A17Rik	2080	2881
NM_018743	LOC55933	2081	2882
AW456595	AW456595	2082	2883
AK020668	1200007B05Rik	2083	2884
NM_007437	Aldh3a2	2084	2885
NM_010437	Hivep2	2085	2886
NM_007706	Cish2	2086	2887

AK017063	4933435A13Rik	2087	2888
AV278924	rij 4933404M19 PX00019F10 1119	2088	2889
NM_008303	Hspe1	1021	1037
AK003228	1110001I14Rik	2089	2890
NM_022880	Slc29a1	2090	2891
AK005033	D7Ertd753e	2091	2892
NM_010497	Idh1	2092	2893
AB051827	Arhu	2093	2894
NM_026172	Decr1	2094	2895
AK014017	Egfr	2095	2896
NM_010324	Got1	2096	2897
NM_011066	Per2	2097	2898
AK004305	D10Ertd749e	2098	2899
AK020922	Pde6h	2099	2900
NM_009381	Thrsp	2100	2901
NM_009016	Raet1a	2101	2902
NM_025545	Aptx	2102	2903
NM_008382	Inhbe	2103	2904
NM_030262	BC003494	2104	2905
BB312353	BB312353	2105	2906
AK007138	2810433K01Rik	2106	2907
AK017354	5430428G01Rik	2107	2908
AK016991	4933430F16Rik	2108	2909
NM_011020	Osp94	2109	2910
NM_019447	Hgfac	2110	2911
NM_020026	B3galt3	1500	1759
AK004138	1110037D04Rik	2111	2912
AK004650	1200008D14Rik	2112	2913
NM_008331	Ifit1	2113	2914
AI551079	Cyp4a12	2114	2915

AK002555	D18Ert240e	2115	2916
NM_025566	2600017J23Rik	2116	2917
AK002477	Tm4sf11	2117	2918
BF322562	Copb1	2118	2919
BB561321	BB561321	2119	2920
AK014658	4833406M21Rik	2120	2921
AK020935	A930036K24Rik	2121	2922
AK004600	Arhgef3	2122	2923
NM_016808	Usp2	2123	2924
NM_015818	Hs6st1	2124	2925
NM_025384	1110003P16Rik	902	957
NM_019781	Pex14	2125	2926
NM_010867	Myom1	2126	2927
AF288783	Pygl	2127	2928
AK008330	2010107C10Rik	2128	2929
NM_008260	Foxa3	2129	2930
NM_010707	Lgals6	2130	2931
AI849720	Ndst1	2131	2932
NM_011967	Psma5	2132	2933
AK003902	1110021L09Rik	2133	2934
NM_009289	Stk2	2134	2935
AK012110	2610511G02Rik	2135	2936
AK010754	2410091N08Rik	2136	2937
NM_032400	Gpr91	2137	2938
AK021023	B430311C09Rik	2138	2939
BB557066	BB557066	2139	2940
BC004781	BC004781	2140	2941
AK004768	Osbp13	2141	2942
NM_025591	2010309E21Rik	2142	2943
AK019783	4930564I24Rik	1515	1774
AK006955	1700080G11Rik	2143	2944

AK013642	2900042M13Rik	2144	2945
NM_023143	Clr	2145	2946
NM_019758	Mtch2-pending	2146	2947
BE691256	2010004B12Rik	2147	2948
BC003488	Lmo4	2148	2949
AK021389	2610511G02Rik	2149	2950
BB463934	1200006P13Rik	2150	2951
AK010472	2410012H22Rik	2151	2952
AK005060	1300019H02Rik	2152	2953
AK004287	1110057L18Rik	2153	2954
AK018458	8430436A10Rik	2154	2955
AK006159	1700020G04Rik	2155	2956
AK004926	Igfals	2156	2957
AK013959	Trim13	2157	2958
AF304306	Hsd17b11	2158	2959
AK004934	1300007L22Rik	2159	2960
AK007710	1810036L03Rik	2160	2961
AV279434	4930458D05Rik	10	60
AK017766	5730512J02Rik	2161	2962
NM_009320	Slc6a6	1521	1780
AK014728	4833419J07Rik	2162	2963
AK014047	3110013K01Rik	2163	2964
BB429858	BB429858	2164	2965
AK011567	2610027H17Rik	2165	2966
NM_030611	Hsd17b5	2166	2967
NM_009444	Tgoln2	2167	2968
AW743226	AW743226	2168	2969
NM_011201	Ptpn1	2169	2970
AK012041	Ris2	2170	2971
AK011544	1500031M22Rik	2171	2972
BB556229	2310015N21Rik	2172	2973

AK014518	Hal	2173	2974
AK020424	9430019C24Rik	2174	2975
AK011578	Pinx1-pending	2175	2976
AK011605	Mrpl45	2176	2977
NM_019992	Brdgl-pending	2177	2978
AK003434	Rbpms	2178	2979
BB131710	BB131710	2179	2980
AK002718	Oprsl	2180	2981
AK009386	2310016F22Rik	2181	2982
NM_017380	9-Sep	2182	2983
NM_007647	Entpd5	2183	2984
NM_009799	Car1	2184	2985
NM_016974	Dbp	2185	2986
AK005032	1300017E09Rik	2186	2987
AK021388	E130114A11Rik	2187	2988
AK003418	1110004G14Rik	2188	2989
NM_021548	Arpp19-pending	2189	2990
AK002217	0610005C13Rik	2190	2991
NM_011825	Prdc-pending	2191	2992
AK005781	1700008N02Rik	2192	2993
AK013950	3110001I22Rik	2193	2994
AK015354	Optn	2194	2995
AK003939	1110028A07Rik	2195	2996
NM_010892	Nek2	2196	2997
AK021082	C030014O09Rik	2197	2998
BB299566	BB299566	2198	2999
AK015050	4930402H24Rik	2199	3000
NM_021507	Sqrdl	2200	3001
NM_023431	9430059D04Rik	2201	3002
NM_023160	Cml1	2202	3003
AK004867	1300002P22Rik	13	63

AK002437	0610009O20Rik	2203	3004
BC006074	1110018G07Rik	2204	3005
AK002772	1500036F01Rik	2205	3006
AK005035	1300017J02Rik	2206	3007
AF241249	1110033G01Rik	2207	3008
AJ131870	Atp2a2	2208	3009
NM_031396	Cnnm1	2209	3010
NM_010189	Fcgrt	2210	3011
NM_011396	Slc22a5	2211	3012
			3013
			3014
AV021580	4922501H04Rik	2212	3015
AK018177	Unc5h2	2213	3016
AK007678	1810033A06Rik	2214	3017
AK004759	1200014F01Rik	1538	1797
AK011406	2610016A03Rik	2215	3018
AK006138	1700019P01Rik	2216	3019
AK012473	2700063E05Rik	2217	3020
NM_031192	Ren1	2218	3021
AV268127	MGC36416	2219	3022
NM_025827	1300002A08Rik	2220	3023
AK010382	2410004E01Rik	2221	3024
AK020283	9130219B18Rik	2222	3025
BB568823	2210414H16Rik	2223	3026
AK004660	Abcd3	2224	3027
AK013812	2900083I11Rik	2225	3028
AK003873	1110020M10Rik	2226	3029
AK012785	Pxf	2227	3030
NM_025661	Ormdl3	2228	3031
AK018462	8430436I03Rik	2229	3032
NM_021304	Abhd1	2230	3033

BC004668	Hps4	2231	3034
M64404	Illm	2232	3035
NM_026232	4933433D23Rik	2233	3036
NM_016669	Crym	2234	3037
BE987053	BE987053	2235	3038
AK015509	4930465M17Rik	2236	3039
AK014531	Palmd	2237	3040
AK018084	6230410J09Rik	2238	3041
NM_023465	Catnbip1	2239	3042
AK011759	2610043O12Rik	2240	3043
AK010209	2310076O21Rik	2241	3044
NM_022985	Awp1-pending	2242	3045
AK016295	4930577M16Rik	2243	3046
AF173639	AI197390	2244	3047
NM_007980	Fabp2	2245	3048
AK002483	0610010I20Rik	908	962
AK021270	C530009C10Rik	2246	3049
AK014111	Hhex	2247	3050
AK007296	1700127B04Rik	2248	3051
AK011417	Pov1	2249	3052
AV378562	2410022M24Rik	1549	1808
NM_010004	Cyp2c40	2250	3053
NM_022983	Edg7	2251	3054
NM_019975	Hpcl-pending	1025	1041
NM_007945	Eps8	1550	1809
AV174028	Bace	2252	3055
AI430696	Peg3	2253	3056
NM_013837	Tpst1	2254	3057
AI266962	Cml1	2255	3058
NM_013484	C2	2256	3059
NM_007994	Fbp2	2257	3060

			3061
			3062
NM_013545	Hcph	2258	3063
AK010430	Ddah1	2259	3064
AK012478	2700063L20Rik	2260	3065
AK008965	Agpat3	2261	3066
NM_013731	Sgk2	2262	3067
AK007574	Fgf21	2263	3068
AK013765	Ecgf1	2264	3069
NM_011933	Decr2	2265	3070
NM_010391	H2-Q10	2266	3071
			3072
			3073
AK004956	1300010F03Rik	2267	3074
AK014740	4833420O05Rik	2268	3075
AK014558	4632408A20Rik	2269	3076
AW120656	MGC28924	1558	1817
AK002851	0610039N19Rik	1559	1818
AK004204	1110048P06Rik	2270	3077
NM_009364	Tfpi2	2271	3078
AV075202	Acadv1	1561	1820
BC003258	BC003323	2272	3079
NM_028094	2010321J07Rik	2273	3080
BB641340	ri A930014C21 PX00066C21 1837	2274	3081
NM_010512	Igf1	2275	3082
			3083
NM_007405	Adcy6	2276	3084
NM_020009	Frap1	2277	3085
AK017403	5430437E11Rik	1565	1824
BC004083	Htatip2	2278	3086

BB229969	BB229969	2279	3087
AV280352	AV280352	21	71
BF532887	ri 6330415L08 PX00008D23 2975	2280	3088
NM_011706	Trpv2	2281	3089
AK009125	2310003N14Rik	2282	3090
AK013267	2810439F02Rik	2283	3091
AK010969	Psm4	2284	3092
AK013874	3010001A07Rik	2285	3093
AK011778	2610100B16Rik	2286	3094
AK017346	Ches1	2287	3095
NM_008796	Pctp	2288	3096
AY004874	Slc23a1	2289	3097
AK009258	2310009O17Rik	2290	3098
AK002859	Aspa	2291	3099
BB483938	AI452195	2292	3100
AK013679	2900053I11Rik	2293	3101
AK017598	5730422A13Rik	2294	3102
AK010891	2510002J07Rik	22	72
NM_010431	Hif1a	2295	3103 3104
AK002480	0610010I13Rik	1574	1833
AK009374	2310016A09Rik	912	966
AK006771	1700052K11Rik	2296	3105
AK016911	4933425E08Rik	2297	3106
NM_007635	Ccng2	2298	3107
NM_010160	Cugbp2	2299	3108
NM_022434	Cyp4f14	2300	3109
AK013725	Dncl1	2301	3110
NM_009824	Cbfa2t3h	2302	3111
AK007630	Cdkn1a	2303	3112

			3113
AK006385	1700026H06Rik	2304	3114
AI875461	AI875461	2305	3115
AK004319	1110059L23Rik	2306	3116
BE990725	BE990725	2307	3117
NM_009362	Tff1	2308	3118
NM_011723	Xdh	2309	3119
NM_010863	Myo1b	2310	3120
AK004905	1300004O04Rik	2311	3121
NM_008391	Irf2	2312	3122
AK014490	3110020O18Rik	2313	3123
AK017615	Sec61a2-pending	2314	3124
AK009820	2310045I24Rik	2315	3125
BB358694	LOC217698	2316	3126
AK002528	Cyp4a10	2317	3127
BB234992	LOC217698	2318	3128
AK010202	2310076L09Rik	2319	3129
AK018164	6330412C24Rik	2320	3130
AK005010	1300015B04Rik	2321	3131
NM_026164	1200006O19Rik	2322	3132
AK005064	1300019I21Rik	2323	3133
NM_008645	Mug1	2324	3134
NM_016915	Pla2g6	2325	3135
NM_030565	BC004044	2326	3136
NM_010255	Gamt	2327	3137
NM_008555	Masp1	2328	3138
BB498227	BB498227	2329	3139
AK011462	2610019F03Rik	2330	3140
BB160481	BB160481	2331	3141
AK018558	9030618K22Rik	2332	3142
AK009057	2310001A20Rik	2333	3143

AK009156	2310004N24Rik	2334	3144
AF377871	Pawr	2335	3145
AK005014	1300015D01Rik	2336	3146
NM_025621	2310050C09Rik	2337	3147
NM_025459	1810015C04Rik	2338	3148
AK009724	2310040G24Rik	2339	3149
BE993937	AI666798	2340	3150
X70514	Nodal	2341	3151
AK020074	6030458C11Rik	2342	3152
AK005383	Pcbp4	2343	3153
AK016973	4833415F11Rik	2344	3154
NM_007865	Dll1	2345	3155
AK009083	Gale	2346	3156
AK012415	2700053F16Rik	2347	3157
NM_013534	Grcb	2348	3158
AV294988	Tacc2	2349	3159
AK010289	2400006N03Rik	2350	3160
AK015259	4930431B09Rik	2351	3161
AK013911	Igsf4	2352	3162
BB157693	BB157693	2353	3163
BF018327	H2-M10.1	2354	3164
AK011266	Gdm1	2355	3165
NM_024240	4933405K01Rik	2356	3166
AK008690	Abhd2	2357	3167
NM_008156	Gpld1	2358	3168
AK006091	1700018L02Rik	2359	3169
AK007264	1700124F02Rik	2360	3170
AK021282	AI848120	2361	3171
AK008072	2010003K11Rik	2362	3172
NM_007954	Es1	2363	3173
AK017446	5530402H23Rik	2364	3174

NM_023207	Wld	2365	3175
BC002253	Al314967	2366	3176
NM_008223	Serpind1	2367	3177
AK009154	2310004N11Rik	2368	3178
AK009435	D17Wsu51e	2369	3179
AK004708	1200011I23Rik	2370	3180
NM_021371	Caln1	2371	3181
AK005346	1500032M05Rik	2372	3182
NM_019687	Slc22a4	2373	3183
AK008038	Slc25a10	2374	3184
AK004692	Sdh1	2375	3185
NM_019867	Ngef	2376	3186
AK007649	1810030A06Rik	2377	3187
NM_010321	Gnmt	2378	3188
AK010239	Fzd7	2379	3189
AK008081	D15Erd747e	2380	3190
AK007644	Dexi	2381	3191
AK012103	Hsd17b12	2382	3192
AK014853	4921509J17Rik	2383	3193
AK010372	2410003M15Rik	2384	3194
NM_011172	Prodh	2385	3195
AK018414	8430415E04Rik	2386	3196
AK015901	MGC28623	2387	3197
BC003470	Pspla1-pending	2388	3198
NM_009040	Rdh6	2389	3199
NM_007972	F10	2390	3200
AK009002	2300002C06Rik	2391	3201
AK005015	Csad	2392	3202
AK007603	1810026B04Rik	2393	3203
AK008844	2210407G14Rik	2394	3204
NM_008295	Hsd3b5	2395	3205

AK021253	C430046K18Rik	2396	3206
AK009918	Cdk3	2397	3207
AK002327	2310075M17Rik	2398	3208
NM_010169	F2r	2399	3209
AW319694	Bucsl	2400	3210
AK014861	4921510J17Rik	2401	3211
NM_008804	Pde9a	2402	3212
NM_018868	Nol5	2403	3213
BB233906	LOC217698	2404	3214
AK003407	1110004C05Rik	2405	3215
BC003974	4933436C10Rik	2406	3216
AJ272272	Psmal	2407	3217
AK014460	3930402G23Rik	2408	3218
NM_009025	Rasa3	2409	3219
AK004971	1300012D20Rik	2410	3220
AK003561	1110008B24Rik	2411	3221
AK020191	8030402F09Rik	2412	3222
AK016678	4933405P16Rik	2413	3223
NM_008655	Gadd45b	2414	3224
AK017918	5830411H19Rik	1614	1873
AK005080	Suclg1	916	970
NM_021314	Tacc2	2415	3225
BB483548	ri C030045D06 PX00075C24 1567	2416	3226
NM_030692	Sacm1l	2417	3227
NM_008086	Gas1	2418	3228
AK019250	2810030D12Rik	2419	3229
AK002889	0610041L09Rik	917	971
BC005585	LOC231086	918	972
AK008206	Snrk	2420	3230
NM_018795	Abcc6	2421	3231

NM_025626	3110001A13Rik	1620	1879
NM_025834	1300015B06Rik	2422	3232
AK004936	Apoa5	2423	3233
NM_011068	Pex11a	2424	3234
AK018684	Hao3	2425	3235
AK017563	5730415C11Rik	2426	3236
AK009450	2310021M12Rik	2427	3237
AK006541	Fac15	2428	3238
NM_020520	Slc25a20	919	973
NM_010172	F7	2429	3239
AK007384	Sult1c1	2430	3240
AK008800	2210402C18Rik	2431	3241
AK010648	2410041F14Rik	2432	3242
AK004920	1300006O23Rik	2433	3243
AK013742	Sca10	2434	3244
AK010922	2510006M18Rik	2435	3245
AK003249	Ppp1r14a	2436	3246
AK016667	4933405K01Rik	2437	3247
AF307987	Ccl21c	2438	3248
AK013918	3100002J04Rik	2439	3249
AK002436	Ran	2440	3250
AK005003	1300014I06Rik	2441	3251
AK009263	2410001H17Rik	2442	3252
AK007239	Meig1	2443	3253
AK009310	Fetub	2444	3254
AK004787	1200015G06Rik	2445	3255
AK003046	Nrn1	2446	3256
AK018565	9030622O22Rik	2447	3257
NM_010702	Lect2	2448	3258
NM_008222	Hccs	2449	3259
AK015368	4930443B20Rik	2450	3260

AK021146	C030044E10Rik	2451	3261
NM_016843	Sca10	2452	3262
AK004540	Arsa	2453	3263
NM_033037	Cdo1	2454	3264
AV252417	AV252417	2455	3265
AK013296	Apex1	2456	3266
AW476218	AW476218	2457	3267
NM_030687	Slc21a5	2458	3268
BB533722	BB533722	2459	3269
NM_019961	Pex3	1030	1046
NM_016763	Hsd17b10	2460	3270
NM_008777	Pah	2461	3271
BF459334	BF459334	2462	3272
AK018358	6820402I19Rik	2463	3273
AK010168	2010004E11Rik	2464	3274
AK011123	Scarb2	2465	3275
BB280678	BB280678	2466	3276
NM_026178	Mmd	2467	3277
NM_012057	Irf5	2468	3278
NM_010476	Hsd17b7	2469	3279
NM_009862	Cdc45l	2470	3280
NM_009266	Sps2	2471	3281
NM_026011	2610313E07Rik	2472	3282
NM_026494	AI413471	1031	1047
NM_009075	Rpia	2473	3283
BB540470	Cyp4a12	2474	3284
BB487754	AI197264	2475	3285
BE991963	Enc1	2476	3286
BC005792	Pte1	922	976
AK014609	4633401B06Rik	2477	3287
AK020260	9030421L11Rik	2478	3288

NM_010422	Hexb	1639	1898
AK013557	2900019G14Rik	2479	3289
AK004798	1200015P04Rik	2480	3290
AB042027	GRSP1	2481	3291
AK012897	Hbb-y	2482	3292
BI556028	ri E130107N23 PX00091H11 1437	2483	3293
AK014530	4933402G07Rik	2484	3294
AK014514	4631408O11Rik	2485	3295
AI450589	0610012F22Rik	2486	3296
NM_008304	Sdc2	2487	3297
AW049168	Dscr111	2488	3298
AK018100	6230429P13Rik	2489	3299
AK011002	Map2k3	2490	3300
AK007964	MGC28885	2491	3301
BC005529	Rin2	2492	3302
NM_008294	Hsd3b4	2493	3303 3304 3305
AV287497	Xnp	2494	3306
AK012712	2810011L15Rik	2495	3307
BF785788	R74766	2496	3308
AK017688	5730469M10Rik	2497	3309
AK007400	Lbh-pending	2498	3310
BB282142	BB282142	2499	3311
NM_011704	Vnn1	2500	3312 3313 3314
NM_013465	Ahsg	2501	3315
NM_015755	Hunk	2502	3316
BC002120	1810013P09Rik	2503	3317

NM_023617	1200011D03Rik	2504	3318
BC003451	LOC232087	2505	3319
AK007392	Ela1	2506	3320
AK016659	4933405A16Rik	1645	1904
AK020614	9530058B02Rik	2507	3321
AK021029	B830003A16Rik	2508	3322
AK010119	Ptpla	2509	3323
AK003844	1110020B03Rik	2510	3324
NM_013797	Slc21a1	2511	3325
NM_016723	Uchl3	2512	3326
BG961761	rij9430029L20 PX00109E05 1 326	2513	3327
NM_010591	Jun	2514	3328 3329 3330
AK012213	Aldh1b1	2515	3331
NM_025964	2310038H17Rik	2516	3332
AK002826	0610039C21Rik	2517	3333 3334
AK004897	Fac12	30	80
NM_011994	Abcd2	2518	3335
AK017296	Ntn3	2519	3336
NM_016928	Tlr5	2520	3337
NM_010776	Mbl2	2521	3338
NM_012006	Cte1	2522	3339 3340 3341
AK002968	0710001L09Rik	2523	3342
AK007645	Gcst	2524	3343
AK012581	0610025L06Rik	2525	3344
AK008702	2210010N10Rik	2526	3345

BI329624	ri 9530008L14 PX00111H18 1536	2527	3346
NM_025768	Grtp1	2528	3347
NM_009624	Adcy9	2529	3348
NM_024223	Crip2	1651	1910
NM_011966	Psma4	2530	3349
AK005897	1700012D01Rik	2531	3350
NM_016748	Ctps	2532	3351
AK017309	Pex1	2533	3352
AK003554	0610008K04Rik	2534	3353
NM_012050	Omd	2535	3354
AK004609	1200006F02Rik	2536	3355
AK007115	1700102P08Rik	2537	3356
NM_013631	Pklr	2538	3357
BB503671	Hsd3b2	2539	3358
AK019762	4930552P12Rik	2540	3359
AK019519	4833432B22Rik	2541	3360
NM_008990	Pvrl2	2542	3361
BB348963	BB348963	2543	3362
AK005546	1600027G01Rik	2544	3363
AK007970	Acf-pending	2545	3364
AK003859	Rtn4	2546	3365 3366 3367
AK017475	5730402C02Rik	2547	3368
NM_023175	D16Ertd502e	2548	3369
AK018142	6330408G06Rik	2549	3370
AK008100	2010004M01Rik	2550	3371
AK002565	Ap3s1	2551	3372
AK003760	1110017O10Rik	2552	3373
BB166389	5730408C10Rik	2553	3374

AK004889	Acadsb	2554	3375
BC002130	Dusp14	2555	3376
NM_023792	Pank	2556	3377
BC003479	LOC216820	35	86
AK003397	1110003P22Rik	2557	3378
AK019381	Pxmp4	2558	3379
NM_007686	Cfi	2559	3380
NM_007976	F5	2560	3381
NM_011375	Siat9	2561	3382
AK018506	8430438D04Rik	2562	3383
AF102849	Haik1-pending	2563	3384
AK008673	2210008K22Rik	2564	3385
NM_011792	Bace	2565	3386
NM_022882	Lpin2	2566	3387
AK015721	4930506M07Rik	2567	3388
NM_019933	Ptpn4	2568	3389
AK011880	2610204K03Rik	2569	3390
NM_018884	Semcap3-pending	2570	3391
AK016577	4932702F08Rik	2571	3392
AK018332	6530411B15Rik	2572	3393
AK017185	5033421K01Rik	2573	3394
NM_011937	Gnpi	2574	3395
AK019527	Wrnip	2575	3396
NM_010062	Dnase2a	2576	3397
AW494273	AW494273	2577	3398
AK008793	2210401N16Rik	2578	3399
NM_010158	Khdrbs3	2579	3400
NM_013565	Itga3	2580	3401
AK009895	Sfrs3	2581	3402
NM_025994	2600015J22Rik	2582	3403
NM_025341	0610041D24Rik	2583	3404

AK013477	1110011E12Rik	2584	3405
AK010387	2410004H02Rik	2585	3406
AK011735	Ppp2r4	2586	3407
NM_007799	Ctse	2587	3408
NM_016689	Aqp3	2588	3409
AK006350	Rasl2-9	2589	3410
AK008555	Pso	2590	3411
AF177211	Gpr105	2591	3412
AK014427	3830408G10Rik	2592	3413
NM_008574	Mcsp	2593	3414
NM_016917	Slc39a1	2594	3415
NM_016918	Nudt5	2595	3416
AB055897	AW413091	2596	3417
AK017223	5133401H06Rik	2597	3418
NM_013697	Ttr	2598	3419
AK003996	1110030O19Rik	2599	3420
AK003495	1110006G02Rik	2600	3421
AK020110	Lbh-pending	2601	3422
AK015173	4930421P07Rik	2602	3423
AK014774	4833426J09Rik	2603	3424
NM_013792	Naglu	1673	1932
NM_008455	Klkb1	2604	3425
NM_019840	Pde4b	2605	3426
NM_011920	Abcg2	2606	3427
AK020473	9430063L05Rik	2607	3428
AC002397	CD4,A-2,B,GNB3, C8,ISOT,TPI,B7,ENO2,DRPL A,U7snRNA,C10,PTPN6,BA P,C2F	1674	1933
NM_019878	Sult1b1	2608	3429
NM_022014	Fn3k	2609	3430

BC002197	C79952	2610	3431
AK002691	D14Ucla2	2611	3432
NM_019877	Copz2	2612	3433
AK017527	5730408K05Rik	2613	3434
AK016217	4930564C03Rik	2614	3435
AK008119	2010005E21Rik	2615	3436
NM_019983	Rab5ef-pending	2616	3437
NM_025597	2700033I16Rik	2617	3438
AK013580	2900024C23Rik	1682	1941
NM_008063	G6pt1	2618	3439
AK002609	0610012J09Rik	2619	3440
BC003725	BC003725	2620	3441
AK020692	Dbi	2621	3442
AK002641	0610016O18Rik	2622	3443
AB042745	Nox4	2623	3444
BE988332	BE988332	2624	3445
AK008235	2010013I23Rik	2625	3446
NM_009900	Clcn2	2626	3447
NM_008639	Mtnr1a	2627	3448
AK020546	9530006C21Rik	2628	3449
AK008532	2610318G18Rik	2629	3450
AK009250	2310009E07Rik	2630	3451
AK010068	D8Ert91e	2631	3452
AK013269	2810439K08Rik	2632	3453
AK002408	0610009I22Rik	2633	3454
AK019969	5730504C04Rik	2634	3455
NM_027853	0610006F02Rik	2635	3456
BC003306	Def8	2636	3457
NM_010501	Ifit3	2637	3458
NM_007494	Ass1	2638	3459
AK008954	2210416J07Rik	2639	3460

AV059994	AV059994	2640	3461
AK010810	2410150I18Rik	2641	3462
NM_009196	Slc16a1	2642	3463
BF682011	Ugp2	2643	3464
AI195543	MGC29978	1033	1049
BE993080	Hsd17b11	2644	3465
M16357	Mup3	2645	3466
M14044	Anxa2	2646	3467
Y10221	Cyp4a12	2647	3468
AA239277	Crot	2648	3469
X01756	Cycs	934	989
BC007172	Galnt2	2649	3470
L02331	Sult1a1	1694	1953
M17818	Mup1	2650	3471
NM_009360	Tfam	2651	3472 3473
BE947329	AW109744	2652	3474
AF009605	Pck1	2653	3475 3476
M21285	Scd1	2654	3477 3478
X53451	Gstp2	2655	3479
X71479	Cyp4a12	2656	3480 3481 3482
BF449960	AW554572	2657	3483
NM_008615	Mod1	2658	3484 3485 3486
W50759	Apoc3	2659	3487
AI648018	2610207I16Rik	936	991

			992
			993
M10022	Cyp1a2	2660	3488
			3489
			3490
U57999	Psap	2661	3491
Z14050	Dci	1034	1050
W54127	Acat1	2662	3492
			3493
			3494
Y09085	Hif1a	2663	3495
AI155095	AI155095	2664	3496
X51397	Myd88	2665	3497
			3498
Y11638	Cyp4a14	2666	3499
			3500
			3501
L33417	Vldlr	2667	3502
AW909415	1110048B16Rik	2668	3503
AJ007749	Casp8	2669	3504
AJ131522	Mlycd	937	1051
			994
AJ011967	Gdf15	2670	3505
M64248	Apoa4	2671	3506
M30697	Abcb1a	2672	3507
AB010826	Cpt1b	2673	3508
			3509
			3510
NM_008342	Igfbp2	2674	3511
			3512
			3513

AW986355	Aco2	2675	3514
AW456981	Mgll	2676	3515
NM_025670	5730403B10Rik	2677	3516
X00945	Spi1-6	2678	3517
X06454	C4	2679	3518
AF072757	Slc27a2	2680	3519 3520 3521
M25944	Car2	2681	3522
M13264	Fabp4	210	215 216 3523
D16215	Fmo1	2682	3524
AF064088	Tieg	2683	3525
NM_013743	Pdk4	42	93 998 94
BC008241	Psmb4	2684	3526
Z71189	Acadv1	939	1001 999 1000
S75207	Hsd11b1	2685	3527 3528 3529
AB033885	Fac14	2686	3530 3531 3532
AA591552	Hsp86-1	2687	3533
AA986766	AA986766	2688	3534
AB003303	Slc10a1	2689	3535
AB006361	Ptgds	2690	3536

AF006688	Acox1	1444	1465 1467 1466
AF007267	Pmm1	1698	1957
AF030343	Ech1	940	1002
AF031814	Nr1i2	2691	3537
AF033196	Rdh5	2692	3538
AF038939	Peg3	2693	3539
AJ001118	Mgll	209	214
D17674	Cyp2c29	2694	3540 3541 3542
D28530	Ptpns	2695	3543
D29016	Fdft1	2696	3544 3545 3546
D86563	Rab4a	2697	3547
J03398	Abcb4	2698	3548 3549 3550
J03549	Cyp2a4	2699	3551 3552 3553
J04696	Gstm2	1703	1962
L20509	Cct3	2700	3554
L31783	Umpk	2701	3555
L47970	Mttp	2702	3556 3557 3558
M16465	S100a10	2703	3559
M21065	Irf1	2704	3560

M21856	Cyp2b10	2705	3561
M27167	Cyp2d10	2706	3562
M29008	Al194696	2707	3563
M29009	Cfh	2708	3564
M31885	Idb1	2709	3565
M64250	Apoa4	2710	3566 3567 3568
M75886	Hsd3b2	2711	3569
M77003	Gpam	2712	3570 3571 3572
M77497	Cyp2f2	2713	3573
M83649	Tnfrsf6	2714	3574
M93275	Adfp	943	1007 1005 1006
U01163	Cpt2	1035	1056 1057
U07159	Acadm	945	1009 1011 1010
U09507	Cdkn1a	2715	3575 3576
U13371	Kdt1	2716	3577
U14332	Il15	1710	1974
U21489	Acadl	946	1014 1012 1013
U23922	Il12rb1	2717	3578
U36993	Cyp7b1	2718	3579

			3580
			3581
U38196	Mpp1	2719	3582
U43298	Lamb3	1711	1975
U47543	Nab2	2720	3583
U48403	Gyk	2721	3584
			3585
			3586
U48420	Gstt2	2722	3587
U58883	Sorbs1	1712	1977
			3588
U59418	Ppp2r5c	2723	3589
U60987	Gdm1	2724	3590
			3591
U79550	Snai2	1714	1979
U83176	Gt(ROSA)26asSor	2725	3592
U89491	Ephx1	2726	3593
X04480	Igf1	2727	3594
X05475	C9	2728	3595
X13135	Fasn	2729	3596
			3597
			3598
X53584	Hsp60	2730	3599
X62940	Tgfb1i4	2731	3600
X70067	Rnps1	2732	3601
X70398	D0H4S114	948	1016
X83971	Fosl2	2733	3602
X89864	Cyp2a5	2734	3603
			3551
			3604
X89998	Hsd17b4	949	1018

			1017
			1019
X96618	Rga	2735	3605
Y14660	Fabp1	2736	3606
			3607
			3608
D87521	Prkdc	2737	3609
M33960	Serpine1	2738	3610
			3611
			3612
AF071315	Cops6	2739	3613
U33557	Fpgs	2740	3614
X95280	G0s2	2741	3615
AB011000	Chkl	2742	3616
AF026073	Sultn	2743	3617
AJ000059	Hyal2	2744	3618
M14757	Abcb1b	2745	3619
M61737	Fsp27	2746	3620
AF075717	TIF2	2747	3621
AI326224	AI326224	2748	3622
J00423	Hprt	2749	3623
			3624
			3625
L23108	Cd36	142	3626
			3627
			3628
X00479	Cyp1a2	2750	3488
			3489
			3490
AI118433	C8a	2751	3629
AI132306	AI132306	2752	3630

AI255955	Il1rap	2753	3631
AI265707	AI265623	2754	3632
AI663818	AI663818	2755	3633
AI854637		2756	3634
AI132665	LOC208677	2757	3635
AI255958	LOC226105	2758	3636
AI266885	AI266885	2759	3637
AI530213	Ugp2	2760	3638
AI461749	AI451155	2761	3639
AI464465		2762	3640
AI503986		2763	3641
D16333	Cpo	2764	3642
X78683	Bcap37	2765	3643
AI482473	Syt14	2766	3644
AI662255	AI662255	2767	3645
AI785285	Dscr111	2768	3646
AI851538	Kcnn2	2769	3647
AB027290	Rab9	2770	3648
AF126798	Fads2	2771	3649 3650 3651
NM_011080	Phxr1	2772	3652
U12790	Hmgcs2	2773	3653 3654 3655
NM_008686	Nfe211	2774	3656
AB017136	Homer2-pending	2775	3657
NM_007843	Defb1	2776	3658
AI647584	AI647584	2777	3659
AW060343	AW060343	2778	3660
AI647917	3200002M13Rik	2779	3661

AI595938	AI595938	2780	3662
NM_010284	Ghr	1728	1994
AW061234	AW061234	2781	3663
NM_008509	Lpl	2782	3664 3665 3666
Z37107	Ephx2	49	101
AI324870	AI324870	2783	3667
X84014	Lama3	2784	3668
Z31362	Npn3	2785	3669
U39066	Map2k6	2786	3670
Z97207	Hspc121-pending	2787	3671
AF161071	Slc2a5	2788	3672 3673
AI646798	AI646798	2789	3674
AF133903	Abcb11	2790	3675 3676
NM_008254	Hmgcl	2791	3677 3678 3679
AF112185	Scnn1a	2792	3680
AI642194	AI463690	2793	3681
AI893641	AI893641	2794	3682
AI596436	AI596436	2795	3683

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.